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A STUDY OF THE BACILLUS (LEPTOTHRIX?) PYOGENES FILIFORMIS (NOV. SPEC.) AND OF ITS PATHOGENIC ACTION.

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PLATES IX–X.

(*From the Pathological Laboratory of the Johns Hopkins University and Hospital.*)

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HISTORY.

A preliminary communication concerning this micro-organism appeared in the *Bulletin of the Johns Hopkins Hospital* for November–December, 1895. The source of the microorganism was one of the laboratory rabbits, of which the following is a brief history:

On March 13, 1895, a large healthy rabbit of the stock of the laboratory gave birth to a litter of young; five days later she was found dead in her cage. The young of this animal died a day or two preceding the death of the mother. The autopsy was performed in the afternoon of the 18th.

The animal was well nourished, and there was no evidence that death had been caused by violence. The mammary glands were still large and contained milk; the peritoneal cavity was smooth and glistening throughout and did not contain an excess of fluid. Upon examination of the abdominal viscera, however, the condition of the uterus arrested atten-

tion. It was several times larger than normal, although much smaller than the uterus of the rabbit at term, and presented a series of dilatations and constrictions which, except for their irregular distribution and peculiar translucency, might have been mistaken for a condition of pregnancy. This possibility was, of course, not to be considered, and, indeed, the dilated and semi-transparent pouches gave the impression of being empty. The serosa covering these dilations was injected, the larger vessels being very prominent and turgidly filled with blood, while the intervening tissue presented a rosy hue. Both cornua of the uterus were similarly affected. Nothing abnormal was observed in connection with the ovaries. The pallor of the interior of the uterus contrasted with the injection of the serous coat. Upon gently stroking the mucosa with a knife a thick, opaque material could be removed, which appeared to be only lightly adherent to the surface of the membrane. It was to the presence of this exudate, extending from the vagina throughout the uterus, that the pallor and opacity of the mucous membrane were due.

In the dilatations before mentioned the mucous membrane was of extreme tenuity, and here the layer of exudate was thinner than elsewhere. The dilatations were apparently produced by the accumulation of gas. After the removal of the exudate the underlying mucosa was found congested.

The pleural cavities contained an excess of fluid of a hæmoglobin-red colour. The serosa itself was covered with a thick, shaggy layer of fibrin-like material. Both the parietal and visceral layers were covered with this material, which was thickest over the diaphragm. The lungs were in part voluminous and in part collapsed, the expanded portions being of firm consistence and apparently consolidated. The whole of the posterior lobe (caudal) was solidified, and the corresponding bronchi contained firm opaque plugs.

The pericardial sac contained a small amount of red fluid between layers of a shaggy exudate similar to that covering the pleuræ.

From the appearance described there could be little question that the death of the animal was caused by an acute pleuritis, pericarditis, and pneumonia, associated with acute endometritis, the presumption being that the causative infectious agent entered through the genital tract. It was thought that the infection had taken place at the time of or soon after parturition.

The bacteriological examination of the exudate contained within the genital tract as well as in the cavities of the thorax confirmed the supposition of infection, and revealed a micro-organism of striking and peculiar morphology. The same organisms were present in the two situations mentioned, although they were more abundant in the uterine cavity.

The discovery of large numbers of micro-organisms in cover-glass preparations led to the preparation of cultures from the different inflammatory exudates. The peculiar morphology of the bacilli to be described awakened the suspicion that it might be difficult, or even impossible, to cultivate them in the usual way and upon the ordinary culture media. Two methods of procedure were therefore employed. In the first place, transplantation of the exudate from the uterus, pleura, and pericardium was made into several kinds of culture media, and these were kept under aerobic and anaerobic conditions. The media employed were as follows:

1, Nutrient gelatin; 2, agar-agar; 3, bouillon; 4, milk; 5, potato; 6, Loeffler's blood serum (from bullock's blood); 7, the same prepared with human blood serum; and 8, the mixture of blood serum, urine, and agar (*Steinschneider*).

Anaerobiosis was obtained by the use of Buchner's jars and by the substitution of hydrogen for air. These culture tubes remained in the thermostat at 37° C. for many days without manifesting any growth whatever. Subsequently attempts were made to cultivate the organism by the use of the coagulated blood and blood serum of the rabbit, but without success. Sterile blood was collected from the femoral artery of the rabbit in Nuttall's bulbs, and after twenty-four hours the separated serum was pipetted off into test tubes. Some of these tubes were heated to 60° C. for thirty minutes, to destroy the bactericidal property of the serum, while the remainder were permitted to remain "active." Bits of the pleural exudate obtained from subsequent cases were transplanted to the blood serum and upon the clot which remained in the bulb after the separation of the serum. Placed in the thermostat, no growth could be detected even after the lapse of two or more weeks. The replacement of the air in the serum tubes by hydrogen did not alter the result. The use of more concentrated media was also tried. Bouillon and agar-agar containing five per cent. of peptone, the other constituents remaining unaltered, were equally unsuccessful, as were also media containing other reducing substances than sugar—namely, formate of sodium (one per cent).

A small bit of the solid exudate obtained from the pleural cavity of the first animal was broken up in sterile salt solution and injected, after a small skin incision had been made, into the right pleural cavity of a second rabbit. The inoculation was positive in its results.

Description of the Organism.

Cover-glass preparations made from the exudate in the uterus show a surprisingly large number of bacilli, which formed a felted network upon the surface. In the interstices between the bacteria a moderate number of mononuclear cells are contained. Cells with more irregular forms of nuclei are more exceptional. The greater part of the solid exudate in this situation is composed of the bacilli; there is considerable variation in the size, and some variation in the appearances of the latter. The size varies from $1{\cdot}4\mu$ to 154μ, which represent the extremes. The majority of the longer forms vary between 56μ and 70μ. The average width of the bacilli is from $0{\cdot}5\mu$ to $0{\cdot}7\mu$. Notwithstanding this great difference in size, the peculiar appearances which we shall describe leave no doubt that only one species is present. The forms of the longer ones, which sometimes reach nearly across the field of the microscope (oil immersion one-twelfth, ocular No. 3) are not straight, but curved. The curves are delicate, long, and sinuous. For the most part the bacilli do not lie in parallel rows. Very few of them in a given field are of the same length or originate from the same point. They frequently cross one another, are, perhaps, curved upon themselves so as to give a looped appearance, and through these differences in direction and form bring about the felting before mentioned. This peculiarity of arrangement, it is believed, is not due to mechanical changes caused by spreading the exudate upon the cover slip, for the lack of uniformity in the thick and undisturbed portions of the preparation is more marked than in the thinner ones, where a rearrangement may have been produced.

Only a very few of the bacilli stain uniformly throughout. For the most part they stain irregularly, and indeed this irregularity of staining is one of their most striking characteristics. It is present

in the small forms as well as in the longest and thus confirms the belief in the similarity of nature of the organisms. The bacilli are composed of longer and shorter strands of protoplasm, which, as compared with the brightly staining points, take on a very faint colour with aniline dyes (Plate IX, Fig. 1). This material forms the matrix in which are placed at regular intervals deeply stained points of chromatin. These deeply staining points, while varying somewhat in size in different bacilli, are, in a given organism, about equidistant from one another. They are cylindrical rather than globular, generally longer than the width of the protoplasmic material in which they lie. The smallest forms contain usually two such brightly staining points, although some contain only one. The chromatin points are never at the ends of the latter, the extremities of the bacilli being composed of the lightly staining protoplasmic envelope. Although the smaller forms may contain only one, they more commonly possess two or three specks, while the longest ones often contain in the neighbourhood of one hundred. The ends of the bacilli are rounded in the large as well as in the small forms, and the former show no evidence of transverse segmentation, which might indicate that they are composed of chains of the smaller forms. There is no evidence whatever that in the uterus the bacteria have been taken up by cells, nor were any appearances observed that indicated a lateral branching.

The bacilli as they appear in the cover slips prepared from the pleura of this case correspond in all essential particulars with those present in the uterus. A few minor points should, however, be noted. There are fewer of the short forms as compared to those in the uterus, although the total number of bacilli is much less than in the latter. This leaves a larger proportion of the longer ones, and indeed this is a striking feature of the exudate in the pleural cavity. The number of solid forms is also greater in this situation, a fact which may be due in part to the albuminous fluid in which they are contained which is rather difficult of decolourization. In the uterus the thickness of the bacilli is distinctive, but in the pleura some variation in this respect is apparent. The protoplasmic strands

are all finer, wider, and the stained chromatin cylinders a little thicker than in the uterus. There is, too, some irregularity in the size, form, and separation of the latter. Altogether there is more variation among the forms in the pleural than in the uterine exudate. The cells which are contained in the exudate are composed of polymorpho-nuclear leucocytes, cells with single oval nuclei and abundance of cell protoplasm and red blood-corpuscles, the last in considerable numbers and sufficient to give to the pleural fluid its red colour. Cells with polymorphous nuclei predominate.

Staining.—The bacilli stain readily in all the aniline dyes. Carbolic-fuchsin and aniline—oil gentian violet (Stirling) stain them very quickly. The irregularity of staining comes out best after treatment of the stained exudate by a 1 to 1,000 watery solution of glacial acetic acid. Without this treatment this feature is much less evident. Stronger bleaching agents (one-per-cent sulphuric acid, Neisser) produce rapid and complete decolourization of the organisms; the use of a solution of Bismarck brown following upon methylene blue, as recommended by Ernst, deprives the chromatic particles of the latter stain. The immersion of the heated cover slips in Delafield's hæmatoxylin for several hours gives a sharp stain of purplish-black colour to the dots and a pale-violet tint to the matrix in which they lie. On the other hand, the bacilli are decolourized upon cover slips both by Gram and by Weigert's methods, and they are not stained by iodine. The fluid which can be collected from the pleural and pericardial cavities contains the bacilli in smaller number than the solid exudate. If a drop of this unheated fluid containing the living bacilli be mixed with a small drop of carbolic-fuchsin or gentian violet and covered with a slip, the chromatin points can be observed to take the stain, leaving the protoplasmic envelope slightly tinted only. The chromatin must therefore be considered to be pre-formed in the manner described within the rods. As C. Fraenkel was enabled by a similar method to discover the branching of the bacillus diphtheriæ, this phenomenon was looked for, but no indication of it was observed.

Motility.—The bacilli are non-motile. Examination of the pleural fluid at once after the death of the animal by means of hanging drops showed entire absence of motility. Bits of the exudate which were broken up in salt solution and examined in this way gave the same result.

Thermal Death Point.—Capillary tubes were filled from the fluid exudate contained within the pleural cavity of an inoculated animal which showed fair numbers of typical bacilli. These tubes were exposed to a temperature of 55° C. for five minutes, and, the contents of several having been mixed, they were introduced into the pleural cavity of another rabbit. No effects resulted. Control animals inoculated directly from the pleural fluid which had not been subjected to this temperature succumbed at the end of a few days. It must therefore be concluded that at the temperature of 55° the bacilli are killed in five minutes, or at least are deprived of all virulence.

Question of Spore Formation and Vitality in Exudates.—The low thermal death point of the organisms indicated that, within the body at least and a short time after death, spores were not formed. The short length of time of their survival outside of the body is indicated by the following experiments, and these would further tend to show that spores are not formed at all. On May 2d a bit of the solid exudate derived from the pleura of an animal which had been autopsied the same day was transferred with the usual precautions to avoid contamination to a recently sterilized test tube. Over the cotton plug a rubber cap was fitted and the tube was put aside in a cupboard exposed to diffuse daylight. On June 18th the tube was examined. Cover-slip preparations prepared from the exudate which was still moist showed typical bacilli, which, however, stained faintly, and besides these the inflammatory cells which now stained very poorly indeed. No other organisms were found, nor were bright unstained sporelike bodies observed. This exudate was broken up in sterile bouillon and injected into the pleural cavity of a half-grown rabbit without any result whatever. The effect of drying upon the bacilli is shown by the following experiment:

On May 26th several bits of the solid exudate derived from the pleural cavity were placed in recently sterilized Petri dishes and slowly dried in the thermostat at 37° C. They remained in the thermostat for forty-eight hours, at the end of which time they were removed and for a short period were exposed to diffuse daylight. Portions of the dried exudate were then powdered and suspended in sterile bouillon. Cover slips prepared from this suspension showed well-staining bacilli. The inoculation of the suspension into the pleural cavity was entirely without effect upon rabbits. The conclusions to be drawn from these experiments are, first, that neither the stained nor the unstained areas in the bacilli are to be regarded as spores; second, that it is very probable that spores are not formed without the body, at least not under the usual conditions of their formation; and, finally, that the vitality of the organism in the outer world is not great.

Experiments upon Animals.

Intrapleural Inoculations.—The failure to cultivate this organism necessitated its transference from animal to animal in order that it might be kept alive sufficiently long to permit the study of its properties. It has already been stated that the inoculation from the pleural exudate of the original rabbit into the pleural cavity of a second one was followed by the death of the latter animal. The conditions which were found in the thorax of the experimental animal were quite indistinguishable from those observed in the original case. Intrapleural inoculations were carried through a series of sixteen rabbits. These covered a period extending from the 19th of March to the 4th of June. Among the rabbits used for these experiments were large full-grown, as well as smaller, one-third- to one-half-grown animals. The amount of material inoculated varied considerably. In all instances the inoculations were made into the right pleural cavity, the precaution always being observed to make a slight skin incision before introducing the needle of the hypodermic syringe into the cavity. As much care as possible was taken not to injure the adjacent lung. The material which was inoculated was

derived in most cases from the pleura of previously inoculated animals, the hæmorrhagic fluid being the part generally chosen. In exceptional cases, however, material containing bacilli derived from the infected subcutaneous tissues, inflamed peritonæum, or visceral abscesses was, after suspension in sterilized fluids, used for the inoculations.

The first of this series of experiments was made on March 19th and the last on June 4th. The period of incubation varied considerably, the longest time observed being five days and the shortest twelve hours. It is, however, necessary to state that the longest incubation was in connection with a large rabbit to which a small quantity of the exudate from the first case had been given, and the shortest a small animal which had received relatively a large amount of the infectious material. There would appear to have been an increase in virulence developed by the successive transference of the bacilli from animal to animal.

Table I.

	Source of infectious material.	Amount inoculated.	Size of rabbit.	Date of inoculation.	Date of death.
1	Uterus of original rabbit.	Bit of the exudate suspended in bouillon.	Large.	March 19, 3 P. M.	March 24, night.
2	Pleural exudate *idem.*	Bit of the exudate.	One-third grown.	*Idem.*	March 24, 9 A. M.
3	Pleural exudate of last.	Bit of the solid exudate.	One-third grown.	March 24, 3 P. M.	March 28, 5 P. M.
4	Pleural exudate.	0·25 c. c. of the hæmorrhagic fluid.	One-half grown.	March 29, 10 A. M.	April 1, 4 P. M.
5	Pleural exudate.	Bit of the solid exudate.	Large.	April 11.	April 14.
6	Pleural exudate.	Small amount of the solid portion.	Large.	April 15, noon.	April 20, 11 P. M.
7	Pleural exudate.	0·25 c. c. of the fluid.	Large.	April 20.	April 23.
8	Œdematous fluid from subcutaneous tissues.	0·2 c. c.	Large.	April 25, 3 P. M.	April 29, night.
9	Pleural exudate.	0·5 c. c. of the fluid.	Large.	April 29, 4 P. M.	May 1, night.
10	Pleural exudate.	0·5 c. c. of the fluid.	Nearly grown.	May 1, 3 P. M.	May 3, 2 P. M.
11	Peritoneal exudate.	A few drops of the fluid.	One-third grown.	May 11, 11 A. M.	May 14, 10 A. M.
12	Peritoneal exudate.	0·33 c. c. of the fluid.	One-third grown.	May 13, 3 P. M.	May 14, 3.30 P. M.
13	Pleural exudate.	0·1 c. c. of the fluid.	Large.	May 21.	May 24.
14	Liver abscess.	Suspension of bit of exudate.	Large.	June 1, 4 P. M.	June 4, night.
15	Pleural exudate.	1 c. c. of the fluid.	One-half grown.	June 4, 5 P. M.	June 5, night.

The table on the preceding page, which gives the dates of inoculation and death, the source of the bacilli, the relative sizes of the animals, and the amount of infectious material employed, is intended to bear upon this point.

The lesions which are produced by the intrapleural inoculation of this organism are so nearly uniform that a separate description of the cases appears hardly necessary. A typical case will be given, and then the more exceptional lesions will be spoken of specifically :

A large male rabbit was inoculated on the 25th of April at 3 P. M. with 0·2 cubic centimetre of the fluid derived from the pleural cavity of an animal autopsied on this date. On April 29th, at 8 A. M., it was found dead.

At the autopsy there was no reaction in the external tissues about the seat of inoculation. The right pleural cavity into which the inoculation had been made was covered with a shaggy, yellowish, pseudo-membranous exudate, forming a uniform covering over the visceral and parietal layers of the pleura, which averaged 1 to 2 millimetres in thickness. The greatest thickness occurred over the diaphragm, where the exudate reached 4 or 5 millimetres in depth. The right lung was compressed, dark red in colour, and completely atelectatic. No distinct pneumonia was visible. A few cubic centimetres of a red, distinctly hæmorrhagic fluid occupied the dependent portions of the cavity. The left pleura did not contain a distinct pseudo-membrane, but it contained an increased quantity of fluid and the surface of the serosa was faintly opaque and covered by a distinctly glutinous material. An exudate similar to that covering the right pleura was present in the pericardium, both layers of which were covered with a fine shaggy, yellowish pseudo-membrane. An increased amount of a faintly reddish serum existed within this cavity. The mediastinal tissues were swollen, congested, and infiltrated with a gelatinous serum, containing bubbles of gas. The exudate on the serous surfaces was readily removed, exposing the congested and inflamed membranes beneath. The remaining viscera, with the exception of the liver, which showed smaller and larger points of yellow, opaque necrosis, were apparently normal.

The exceptional cases were, in the first place, those in which the animals succumbed in from twelve to twenty-four hours, in which instances the solid exudate in the pleural cavity, although much reduced in amount, was still present. The transuded fluid was never absent and it always presented the reddish colour previously mentioned. As early as twelve hours after the inoculation there was evidence of a great increase in the organisms introduced. In another instance, in addition to the typical lesions which occurred in the

pleura and pericardium, an abscess as large as a split pea was present in the wall of the left ventricle. It was opaque and firm and involved the entire thickness of the myocardium. In a further small number of cases the extension of the inflammatory process from the pleura could be followed into the peritoneal cavity, where it was localized upon the cephalic surface of the liver, corresponding to the part in contact with the surface of the diaphragm. The pseudomembrane present here sometimes reached a size 3×3 centimetres in extent. It was thin and opaque, and after its removal, which was easily accomplished, the capsule of the liver appeared thickened and opaque. An exceptional instance of extension to the peritonæum with the production of a general peritonitis is the following:

A small one-third grown rabbit received into its pleural cavity on May 13th, at 3 P. M., 0·33 cubic centimetre of the peritoneal fluid from a case of peritoneal infection, which was autopsied the same day. It was dead at 3.30 P. M. May 14th.

The right pleura, into which the inoculation had been made, presented a pinkish colour and contained a small excess of fluid and a few flakes of fibrin adherent to its surface. This fibrin was found upon examination to contain the typical micro-organisms. Neither the opposite pleura nor the pericardium was involved. The peritoneal cavity contained an excess of fluid. The intestines were moderately distended and small flakes of a fibrinlike material were present on the omentum and the mesentery. Covering the right side of the liver there was a thick exudate. On section of the liver, this was seen to correspond with areas of smaller and larger abscess formation which occupied both the caudal and cephalic lobes of this side. The false membrane was continuous over the parts of the liver in which the abscesses were contained; it was scattered as small foci in other parts.

There can be, I think, little doubt that the peritonitis in this instance followed the extension of the liver abscesses to the capsule of the liver. Moreover, it illustrates the fact that such profound pathological lesions as these, associated with a great increase in the number of bacilli, can take place in so short a time as twenty-four hours.

In two instances, through some fault in the technique employed, the material containing the bacilli came into contact with the soft tissues over the thorax as well as with the pleural cavity. In one of these cases there was an inflammatory, œdematous exudate extending

over a considerable part of the thoracic wall and containing bubbles of gas. In the second the subcutaneous tissues and muscle were densely infiltrated with a thick, yellowish, firm material, which rendered the tissues hard and boardlike, the involved muscle appearing opaque and necrotic.

Pneumonia as a complication of the pleural cases occurred in several instances. It was present, it will be recalled, in the spontaneous case involving the caudal lobe of the right lung; in the experimental cases it was limited exclusively to the right side and usually involved less than an entire lobe. In one instance what appeared to have been an extension from the lung tissue to the bronchi was observed, and in this one the main bronchus proceeding to the affected lobe was plugged by a firm exudate.

The intrapleural inoculation of guinea-pigs was also followed by positive results. The following is a typical case:

A guinea-pig was inoculated into the pleural cavity at 4 P. M. on April 20th with 0·33 cubic centimetre of the pleural fluid derived from a rabbit dead the same day. The animal was observed to be ill on the morning of the 21st and was found dead on the 22d at 7 A. M.

The autopsy showed entire absence of reaction about the seat of inoculation. The right pleura was covered with a sticky, tolerably thick, opaque, greenish-white, slightly adherent pseudo-membrane. The lung was compressed, apparently free from consolidation, and an increased quantity of fluid was present in the pleural cavity. The exo-pericardium was covered with a thinner exudate than the lung, and its inner surface was injected, the sac itself containing turbid fluid. A thin layer of exudate covered the left pleura. The lung on this side was not compressed, although there was an increased amount of fluid in the pleural cavity. The liver showed several superficial small necroses. Cover slips prepared from the solid and fluid exudate exhibited typical bacilli, among which there were both polymorpho-nuclear and mononuclear cells. It was noticed in this case that a small number of the bacilli had been taken up by leucocytes. The long forms, which often exceeded the size of the pus corpuscles in which they were contained, were curved so as to accommodate themselves to the size of the phagocyte. The contained bacilli stained for the most part more feebly than those outside. In one instance a bacillus was seen half within and half without the corpuscle. The part within stained more feebly than the part without.

Intraperitoneal Inoculations.—In no instance did an animal survive intrapleural inoculation. It is interesting, therefore, to note

that inoculations into the peritonæum, although generally successful, failed in some cases and that the period of incubation was at times longer than in the pleural cases.

On April 20th a large rabbit received intraperitoneally 0·33 cubic centimetre of the pleural fluid from a rabbit dead that day. It was found dead on the morning of the 25th.

At the autopsy the tissues over the ventral (inferior) surface of the body were enormously swollen. The swelling affected particularly the subcutaneous tissues extending from the mid-portion of the thorax almost to the pubes and laterally to the axillary line. The infiltrated tissues were fully 1 centimetre in thickness; they were gelatinous and blood-stained. The muscles appeared to have escaped. In the superficial layers the subcutaneous tissue was intimately infiltrated with fluid, which manifested little tendency to escape, whereas in the deeper parts just above the muscles the tissues were swollen and necrotic. Gas bubbles were present both in the superficial œdematous tissue and in the deeper necrotic parts. Although the muscles just beneath the superficial fascia appeared to have escaped, the recti on both sides were found to have been converted into a yellowish necrotic material of almost stony hardness. The necrosis extended through the entire muscle and the subserous tissues, and, corresponding to the latter, an acute exudate covered the peritoneal coats. The peritonitis thus described involved the lower portion of the right lobe of the liver and that part of the cæcum which is situated immediately below the right lobe of the liver, where the exudate reached 1 millimetre in thickness.

Cover-slip preparations were made from the subcutaneous œdema, the necrotic muscle, the localized peritonitis, and from the general peritoneal cavity. The bacilli were present in all situations excepting the last.

The interpretation of this case is not entirely clear. It must at least be considered that the inoculation may have failed to reach the peritoneal cavity, that it entered only the subcutaneous tissues over the abdomen, and that subsequently an extension to the peritonæum took place from the necrotic abdominal muscles. While the reverse infection can not be positively excluded, it is, we think, hardly probable, particularly as we expect to show that the subcutaneous inoculations lead in themselves to extensive necrotic conditions of the soft tissues.

On May 8th a large rabbit received into the peritoneal cavity 0·33 cubic centimetre of the pleural fluid of a previous rabbit. The animal was dead in forty-eight hours.

The peritoneal cavity contained a considerable quantity of sticky fluid and large fibrinous flakes. The greatest accumulation both of fluid and

fibrin was in the pelvis, although there was a rich accumulation of lymph between the liver and the diaphragm. The serosa generally was injected and small bits of fibrin were attached to various parts of the visceral and parietal layers. The fluid portion of the exudate presented a distinctly glutinous character.

Cover-slip preparations showed many of the characteristic bacilli.

This case is an example of a pure peritoneal infection with rapid termination, while the following one is an example of long incubation:

On April 29th a large rabbit received 0·5 cubic centimetre of the pleural fluid of a previous animal. It was found dead on the 6th of May, having in the meantime lost some in weight.

About the point of inoculation there was some œdema of the subcutaneous tissues. The peritonæum did not show a uniform distribution of the inflammatory exudate. In the lower zone of the abdomen a small collection of sticky fluid existed, whereas small white masses were scattered at various places over the parietal and visceral peritonæum and the mesentery and omentum, the larger masses occurring between the loops of the intestine. The blood vessels of the serous membrane were injected. Between the diaphragm and the liver the pseudo-membrane was thickest. The under surface of the diaphragm appeared inflamed, both pleural cavities contained a sticky fluid, and the mediastinal tissues were infiltrated and œdematous.

Cover-slip preparations from the peritoneal cavity, from the pleural cavity, and from the mediastinal tissues showed the typical organisms.

The last two cases show that this organism, when introduced directly into the peritoneal cavity, is capable of setting up an acute, fatal peritonitis. It is interesting to note that in the last case an extension of the process—probably through the diaphragm, possibly through the mediastinal tissues—into the pleura occurred.

Intrauterine Inoculation.—A pregnant rabbit was laparotomized on May 11th, at 3.30 P. M., and received 0·33 cubic centimetre of a faintly turbid suspension, prepared from a fibrinous flake obtained from the peritoneal exudate, directly into one of the dilated segments of the pregnant uterus. The inoculated fluid had been examined by means of cover-slip preparations, and it was observed to contain very few of the micro-organisms. The wound in the abdominal walls was closed with silk sutures—first the peritonæum, then the skin, and the whole covered with a celloidin dressing. Strict antiseptic precautions were maintained throughout. The animal quickly recovered from the anæsthetic, but a hernia soon developed. Death occurred on May 13th, at 1.30 P. M.

At the autopsy the skin incision was found intact. There was no sup-

puration. A small knuckle of gut, covered with omentum, protruded through the lower angle of the wound and lay between the peritonæum and the subcutaneous tissues. The tissues covering the symphysis pubis and in the inguinal regions were emphysematous and œdematous. It may be mentioned that in this fluid no micro-organisms could be detected upon the cover slips. On removing the omentum over the protruded loop of intestine, small hæmorrhages were observed beneath the serosa, covering some of which there was a deposit of yellowish fibrin. Other small hæmorrhages occurred in the serosa of the large intestine. The peritoneal cavity itself contained an excess of bloody fluid, similar to that seen in previous cases in the pleural cavity. In the more dependent parts a thin, puslike exudate had collected. The segment of the uterus into which the injection was made was much congested, and was beset with small hæmorrhages. The remaining segments of the right side (side of inoculation) were congested, while on the left side they had collapsed.

Upon opening the uterus, one fœtus only was found, and this corresponded with the segment into which the inoculation had been made. It was suffused with blood, dark in colour, and covered with a grayish, thin exudate, which also covered the mucous membrane unoccupied by fœtal structures. The left cornu of the uterus was more dilated, notwithstanding its collapsed condition, than the right. It contained a thin, opaque, sanguinolent fluid in considerable amount. What appeared to be remains of fœtal structure were found, although, with the exception of the one fœtus described, no others were present within the uterus.

The examination by means of cover slips of the fibrin and fluid in the peritoneal cavity, as well as the contents of both cornua of the uterus, gave typical bacilli, varying somewhat in numbers in different parts.

The effect of this inoculation was, undoubtedly, to cause an abortion in the animal, inasmuch as, at the time of the operation, the various dilated segments of the uterus were observed to contain fœtuses. Notwithstanding precautions which were taken to avoid infection of the peritonæum, an acute peritonitis due to the bacillus pyogenes filiformis developed. In a previous instance an attempt had been made to inoculate directly into the pregnant uterus from the vagina, but without success. It was thought that by this means it might be possible to reproduce the precise chain of events which were supposed to have occurred in the original animal, but, as there is a great probability that the infectious material never entered the uterus at all, the failure of this experiment was not unlooked for. In the present instance of direct inoculation through the laparotomized peritonæum partial success only was achieved. The pleura

and pericardium, as well as all the extraperitoneal viscera, escaped infection.

Meningeal Inoculations.—On April 25th, at 3.30 P. M., a full-grown rabbit was anæsthetized, and, after trephining over the mid-portion of the skull, a bit of the exudate, derived from the subcutaneous tissues of another rabbit autopsied the same day, was introduced with antiseptic precautions beneath the dura mater. No immediate effect was produced by the operation. The animal died at 2 P. M. on April 25th, thus having lived twenty-two and a half hours.

Upon the removal of the celloidin dressing and the separation of the skin over the trephined opening, there was an escape of pus mixed with gas bubbles from the wound. Cover-slip preparations, prepared from this material, showed it to contain the introduced bacilli in pure culture. The tissues of the scalp over the side of the incision were œdematous over a considerable area, and contained the characteristic bacilli. The meninges covering the convexity of the brain were hyperæmic, and here and there ecchymotic. They were also somewhat œdematous. On the left side of the brain, about its mid-portion and 3 millimetres from the superior longitudinal fissure, an elevated excrescence 1·5 millimetre in extent was found. This corresponded with the trephined area and the seat of inoculation, and it was evident the inflammation had spread from this point.

Cover-slip preparations from the various portions of the cerebral meninges showed the typical organisms in varying numbers.

After hardening the brain in alcohol, an incision carried through the protuberance mentioned corresponded with a cavity in the cortical substance about the size of a split pea, the walls of which were smooth and which could readily have been made by the accumulation of a gas. A second rabbit, which had been inoculated in a manner similar to the last one, succumbed in about twelve hours. The lesions found at autopsy were similar to those already described, although they were perhaps less marked. Œdema and gas formation were found in the tissues about the trephined opening; beneath the dura a small collection of pus corresponded to the site of the inoculation, and from this point the remainder of the meninges over the convexity were infiltrated with pus and serum. Only microorganisms having the appearance of those introduced at the operation were present. In neither case was there any development of metastatic inflammatory processes in other parts of the body.

Subcutaneous Inoculation.—In connection with one of the cited cases, in which the inoculation was made into the peritonæum, it

will be recalled that there was considerable involvement of the subcutaneous tissues and muscles of the abdominal walls. That fatal results may follow purely subcutaneous inoculation is shown by the following case:

A large white rabbit was inoculated into the subcutaneous tissues and muscles of the right side of the thorax. Corresponding with these tissues there was present at the autopsy a very extensive opaque, yellowish-white, rather dry necrosis and exudation, involving the tissues from the skin nearly to the bone. The involved tissues occupied a large, irregular area, beginning near the middle line in front and extending over the right side of the thoracic wall nearly to a line drawn from the axilla to the crest of the ilium, and reaching 6 to 7 centimetres in length. Surrounding this sharply defined but rather irregularly outlined necrotic area there was a moderate gelatinous œdema with occasional dark-red hæmorrhages. The involved muscle was firm, infiltrated, and showed an opaque, whitish necrosis.

Cover-slip preparations prepared from the necrotic tissue showed large numbers of the characteristic pleomorphic bacilli. The remaining viscera were free from localization of the organism.

Intravenous Inoculations.—Perhaps the most interesting, certainly the most widespread, results were obtained by the intravenous inoculations of the pleural fluid. The results were uniformly fatal, the animals succumbing in from two to four days after the inoculation. At autopsy, abscesses were present in the viscera. These were generally miliary in size, although at times they were larger and spreading. Preference was exhibited in reference to their localization, certain organs being entirely spared. The abscesses were never absent from the brain and heart muscle; they appeared occasionally in the liver, more rarely still in the voluntary muscles, never in the kidneys, spleen, or lungs.

On May 8th, at 10 A. M., a grown rabbit received 0·16 cubic centimetre of the pleural fluid into the ear vein. The animal died May 11th, at 10 A. M.

At the autopsy there was an increased amount of fluid of a sticky character in the peritoneal cavity which contained the characteristic organisms. On both surfaces of the peritonæum fibrin flakes were present. The liver was especially remarkable, as it was covered by a pseudo-membrane, which in part was thick and opaque. The membrane was present in greatest amount over the caudal lobe just above the stomach. Upon incision of the liver, it was found to be the seat of many abscess formations, the largest

occupying the caudal lobe mentioned. Except in the latter situation the abscesses were small, whereas in this lobe a large abscess, involving its entire thickness and almost its entire extent, existed. Apparently it was produced by the coalescence of many of the smaller abscesses. As these were frequently situated in this part of the liver as well as in others directly beneath the capsule, the extension of the inflammatory process to the peritonæum probably took place at these points. The heart muscle contained a few abscesses. A very few were present also in the substance of the brain. None occurred in the kidneys, spleen, lungs, or voluntary muscles.

The effects of the intravenous inoculations with respect to the point of localization of the bacilli were in part determined by the local conditions. For example, the inoculation into non-pregnant female animals was not followed by the reappearance of the micro-organisms in any of the structures of the genital tract, whereas in the case of pregnant animals the inoculations were followed by the localization of the organisms and the inflammatory process in the pregnant uterus. Again, the occurrence of previous disease—as, for example, psorospermosis in the liver of the rabbit—determined, apparently, the localization of the infectious material with the production of large and spreading abscesses in this viscus.

On April 29th, at 4.30 P. M., a pregnant animal received 0·5 cubic centimetre of the pleural fluid into the marginal ear vein. There were no immediate effects. On the morning of May 1st the rabbit was observed to be in convulsions. At 8.30, at which time I saw it, it was lying on its left side, with its spine curved and its head drawn back, until it touched the vertebral column. Slight movements or noise brought on severe convulsions. The latter usually began in the extremities, but rapidly extended over and involved the whole of the body. Placed on its right side, the animal soon returned to the left, although the convulsions made this difficult to accomplish. The eyes converged to the inside. The convulsions continued to occur from time to time during the day, the animal growing perceptibly weaker. There was subnormal temperature before death. Measured in the rectum at 2 P. M., the thermometer registered 32° C. The animal was found dead at 8 A. M. on May 2d. It was cold and stiff, and had evidently been dead some hours.

Autopsy.—The brain showed many abscesses. They averaged the size of a hemp seed, and were situated in both hemispheres and in the frontal, temporal, and sphenoidal regions. They were not absent from the base of the brain, although, apparently, they were most numerous in the cerebral cortex, a single one only having been discovered in the left lateral lobe of the cerebellum. On section of the alcohol-hardened brain, similar abscesses

were found in the depth of the white substance. The meninges were injected and the cerebral vessels dilated. The spinal cord showed no macroscopic alterations. Cover-slip preparations from the meninges gave a few bacilli, whereas from the brain abscesses the greatest number of characteristic and beaded forms were obtained. The heart muscle contained several abscesses. The largest occupied the wall of the left ventricle, several smaller ones, the size of miliary tubercles, occurring in other situations. Cover-slip preparations from these showed the characteristic bacilli.

The Uterus.—Several fœtuses, averaging 4 millimetres in size, were contained in the two cornua of the uterus. Of these, two only were examined. They were found to be hæmorrhagic, and an extravasation of blood had taken place in the membranes surrounding them. The placentæ were not hæmorrhagic. The cavity of the uterus throughout, which was not occupied by the fœtal structures, was covered with a gray or yellowish exudate, and several of the uterine segments which contained young were distended with gas. The condition observed here reminded one strongly of the appearances first noted in connection with the original case. Cover-slip preparations prepared from various parts of the uterine cavity showed the characteristic bacilli. They were, however, most numerous in the grayish exudate which covered the mucosa.

On May 1st, at 3.30 P. M., a grown rabbit received 0·5 cubic centimetre of the pleural fluid intravenously. Death occurred May 4th at 12 M. Convulsions were not noted in this animal.

At the autopsy the brain presented much the same appearance as in the previous one. There were, however, in addition, distinct abscesses in the olfactory lobes. The heart presented an extraordinary appearance. Through the unopened pericardial sac large numbers of abscesses were observed in both ventricular walls. These were situated below the epicardium, which was hyperæmic and covered with a sticky fluid. In the substance of the heart muscle and in the papillary muscles beneath the endocardium the abscesses averaged in size a mustard seed. They were yellow in colour and of firm consistence. One hundred and sixty were counted beneath the epicardium and the endocardium without taking into consideration those in the muscular substance itself. The liver in this case contained psorosperm nodules. In the substance of the liver there occurred a number of abscesses which in the posterior or caudal lobe reached a large size and were surrounded by a zone of necrotic liver substance. A localized peritonitis was present in the liver over the affected areas. The kidneys, spleen, uterus (non-pregnant), voluntary muscles, and joints were free from localizations of the organism. The bacterioscopical examination showed large numbers of bacilli in the substance of the abscesses.

With the exception of the instances of general infection, to be mentioned later, in no case of intravenous inoculation were abscesses absent from the brain and heart muscle. The number of abscesses

present in these situations varied in the different cases, although, as a rule, they were present in large rather than in small numbers. In one case a large spreading abscess occupied the psoas and iliacus muscles on the left side, involving almost the whole of these structures and extending into the adjacent soft tissues.

In no instance did abscesses appear in the kidneys, the spleen, the lungs, or the joints, nor in the genital organs, excepting in cases of pregnancy, as has already been mentioned. As compared, therefore, with experimental staphylococcus-aureus infections, these differences in localization are to be remarked. It will be recalled that in the latter abscesses are, if present anywhere else, rarely absent from the kidneys; they are not infrequently present in the heart and the voluntary muscles, less commonly in the central nervous system and other organs. It does not seem possible, therefore, to explain these differences by the facts regarding the circulation in the different organs. We incline to the view, of which we shall have more to say later, that the different organs present varying opportunities for the development of bacteria even during life, owing to the peculiar metabolism with which they are concerned, and which must modify their actual chemical composition.

In the cases previously mentioned, cover slips were made from organs which gave no evidence of the localization within them of the bacilli, and these were found uniformly not to contain the organisms. In no instance was a single bacillus met with in these preparations. In consequence of our inability to cultivate the bacillus, the study of these cases by cultures, which would have been more satisfactory and conclusive, could not be carried out.

That this organism is capable of producing actual septicæmia is proved by the following case; but, while it does not stand alone as the one instance in which a general infection was produced experimentally, it is to be regarded as exceptional in this respect:

On June 8th, at 12 M., a half-grown rabbit received 0·5 cubic centimetre of the pleural fluid of another rabbit autopsied the same day into the marginal ear vein, and succumbed during the night.

At the autopsy, which was made on June 9th, at 9 A. M., there was no excess of fluid nor any inflammatory exudate present in the peritoneal cavity. The spleen was enlarged, softened, and congested. The liver was congested and contained many superficial and deep foci of necrosis. The kidneys were swollen and the lungs congested. The pleural cavities contained a small amount of bloody fluid, the pericardium a similar fluid, while the brain was apparently normal.

Cover-slip preparations from the spleen, liver, kidneys, lungs, pleural and pericardial fluids, the heart's blood, bile, and brain—all showed large numbers of typical bacilli.

Inoculations which failed.—From time to time while the experiments described above were being conducted inoculations of the same material were made into other animals with negative results. It will be recalled that in the guinea-pig the intrapleural inoculation was successful. On the other hand, the subcutaneous injection of considerable quantities of the infectious material into these animals produced not the slightest reaction, nor did they give rise, apparently, to any inconvenience to these animals. The inoculation of relatively large amounts subcutaneously or intrapleurally into mice, whether wild or tame, were never followed by any apparent effect. Pigeons likewise showed themselves practically indifferent to the subcutaneous, intramuscular, and intraperitoneal injections of quite large quantities of the infectious material. One of the subcutaneous veins of the leg was exposed in a dog into which 1 cubic centimetre of peritoneal fluid containing many bacilli was injected. There was no immediate effect following the injection, nor did any perceptible untoward symptom develop during several weeks of observation of this animal. When we consider the great susceptibility of the rabbit, and the fact that there appeared only slight and insignificant differences in the mode of its reaction to inoculations conducted in various ways, the variations in the case of the guinea-pig appear the more remarkable, and the entire absence of susceptibility in the remaining animals which were tested worthy of remark.

The Organism as obtained from the Experimental Cases.

In the course of the transference of the infected material from animal to animal certain variations in the appearance of the organ-

isms as they were derived from different cases were noted. These variations were never so great as to warrant the supposition that the original species was lost, or even had become, in the course of its transference, admixed with another species. The variations affected the relative numbers of short forms and long forms which might occur in any given situation, and, furthermore, the degrees to which the irregular staining, already described as characteristic of this organism, became less regular or tended entirely to disappear. For example, in the course of the successive inoculations into the pleura it was noted that the short forms of the bacilli diminished greatly in numbers, and whereas in the beginning very few solidly staining forms occurred, as time went on a larger number of non-beaded ones appeared. The total number of the latter was, however, never so great as was observed under some other conditions, as, for example, in certain cases of intravenous inoculation and in some situations in the infected uterus in the case of the intra-uterine inoculation. Closely allied with the tendency to appear as solid forms was the tendency toward increased thickness in the latter. Now and then the solid bacilli reached a thickness of at least twice that of the characteristic forms. It would further appear, from an observation made in the case of the intra-uterine inoculation, that the solid forms really represented stages toward degeneration. In this instance positive evidences of involution were obtained in those bacilli which had lost their characteristic markings. These were swollen to twice the average size and contained irregular vacuoles. In extreme cases the chromatin material was irregularly segmented and stained with varying degrees of intensity, and an appearance of knoblike projections was noted in certain of the bacilli. The latter were present in the largest number in the cases of abscesses developed after intravenous inoculation. They gave a peculiar appearance to the modified organisms, inasmuch as they were composed of very deeply staining protoplasmic masses situated at irregular intervals along the course of the rods. The swellings averaged about twice the thickness of the rods, and might be of even still greater size. They were not to be confounded with appearances

brought about by angular bends and by an irregular twisting to which the longer bacilli were more or less subject, and which might occur several times in the course of the longer forms. From the study of certain of the larger abscess formations—as, for example, in the subcutaneous tissues and in the liver—the impression was obtained that the size of the bacilli varied to a certain extent with the number present and the rapidity of their development. In the contents of the abscesses in both of these places the numbers were very great indeed, and coincidently the shorter forms, which had more or less disappeared, now reappeared, and rather predominated over the larger ones. The changes in staining properties and those attributable to involution were not noted in the smaller forms.

Phagocytosis.

Although in all the local exudates the bacilli were associated with pus corpuscles, the phenomenon of phagocytosis was observed in a few instances only. In the case of meningeal inoculation in which the animal lived twenty-two hours and a half a great emigration of leucocytes had occurred, and very occasionally the bacilli which for the most part stained faintly were detected in polymorpho-nuclear leucocytes. Such phenomena were very unusual in the pleura, although in a very few instances cell inclusions had taken place. In one case of intraperitoneal inoculation, in which death occurred after forty-eight hours, bacilli were observed occasionally, both within the cell protoplasm and within the nuclei of the emigrated white corpuscles. The best example of phagocytosis was observed in a case of intra-pleural inoculation of the guinea-pig, in which death occurred in about forty hours. The inflammatory exudate contained a large number of leucocytes with polymorphous nuclei, and these not infrequently included bacilli. The included forms were for the most part long, and they were curved round the interior of the cell, owing to the fact that they were often several times as long as the cell itself. The included organisms stained, as a rule, more faintly than those without cells, although there were not a few exceptions to this rule. The phagocytic pus cells appeared quite normal, and contained

bright, well-staining nuclei. It has already been mentioned that in one instance an imperfectly inclosed bacillus was observed, in which the intracellular stained more faintly than the extracellular portion.

Cultivation of the Organism.

Notwithstanding the failures to cultivate this bacillus which have been mentioned in detail in the early part of the paper, further attempts were made upon the ordinary media from time to time in the course of the transference of the organisms from animal to animal, and always with the same negative results. In no instance was the slightest evidence of increase of the bacilli obtained. At this juncture it was decided to attempt their growth upon the sterile organs of the rabbit itself. With this end in view, experiments were first conducted for the purpose of determining whether or not it would be possible to isolate the various organs of the rabbit and preserve them free from contamination with extraneous micro-organisms. Very little difficulty was experienced in this direction. The hair of a rabbit, which had been killed by a sharp blow on the back of the neck, was laid by wetting it thoroughly with ninety-five-per-cent alcohol. The skin was now cut through with sterile and heated scissors, and dissected back with sterile instruments, leaving the abdominal walls, minus the skin and subcutaneous tissues, widely exposed. The latter were flushed with alcohol, a sufficient excess being used to moisten thoroughly the surface and to form small accumulation in the angles formed at the point of junction of the abdominal walls and the dissected skin. By means of instruments rendered sterile with heat the abdominal parietes were now laid back, care being exercised to avoid even contact with underlying intestines. Recently sterilized test tubes of larger calibre than those usually employed, and Petri's dishes, also recently sterilized, being close at hand, pieces of the liver, spleen, kidney, uterus, and lung were quickly excised with sterile instruments and dropped into these vessels. The heart was removed with its pericardial investment entire and placed in a Petri plate. Care was taken to avoid contact with the upper portions of the test tubes to which the cotton plug was ap-

plied. The charged plates and tubes were now placed aside for several days, and those which remained uncontaminated were inoculated with material derived from the experimental animals. It is interesting to note that only occasionally did one of these tubes show any contamination. Inoculations were made upon the sterile organs on April 2d, and the cultures, placed in the thermostat at the temperature of 37° C., were removed on the 5th of April. One of the lung tubes was found to have become contaminated by a growth of mould, and was discarded. The tubes containing the liver and the spleen had assumed a slightly opaque appearance. They were, however, entirely free from odour of putrefaction, and cover slips which were prepared showed an entire absence of micro-organisms, including even the transplanted bacilli. One of the kidney tubes was likewise free from organisms of any kind, although it had been inoculated with the bacillus pyogenes filiformis with the rest. A Petri plate containing a whole kidney, which had been incised in the middle and allowed to fall apart, the freshly cut surface of which had been inoculated, showed a faintly opaque, slightly greenish, elevated deposit, which, upon examination, proved to be a growth of micro-organisms similar to those which had been transplanted.

The inoculation of the heart was made between the layers of the pericardium. On removal from the thermostat there was an entire absence of odour, the organ looked only a little darker than at the time of its introduction, a similar opacity to that present on the kidney had spread over the limits of the pericardial sac, and cover-slip preparations showed a growth of organisms resembling those observed in connection with the kidney.

The inoculations of the uterus were made directly into its cavity. Removed from the thermostat, it presented a slightly greenish appearance, possessed a very faint odour—not, however, one suggesting putrefaction—and an opaque material covered the exposed ends of the inoculated tissue. Cover-slip preparations of the latter showed the same organisms as upon the two previously mentioned organs.

The previous descriptions refer to cultures under ordinary conditions. Similar ones were made in an atmosphere of hydrogen. The

anaerobic tube containing the liver showed a considerable separation of bloody fluid, but there was absolutely no development of the introduced bacilli. The surface of the lung in anaerobic culture was moist and grayish in appearance and contained numerous bacilli indistinguishable from those observed in the aerobic cultures. It can, however, be said that the growth was less abundant in the anaerobic tubes than in those exposed freely to the air. Thus it will be observed that upon the sterile organs of the rabbit the first success of our many attempts to cultivate this organism was met with.

Transplantations were now made from organ to organ, and subsequent generations were obtained—not, however, with equal success in all cases. For example, in the case of the lung a new growth appeared, but the organisms could not be grown upon this medium beyond the third generation. The uterus also permitted the development of three generations only, whereas in the case of the kidney a second generation, but not a third, was obtained. The first growth upon the organs was the most vigorous, the subsequent generations being very slight indeed, and the last hardly to be recognised by the unaided eye, but requiring the use of cover-slip preparations to demonstrate the presence and increase of the transplanted micro-organisms. On April 20th the experiments were repeated, but this time a pregnant female rabbit was employed for obtaining the material for cultures, and to those organs previously used portions of the sterile placenta were now added. The result of the inoculation of a full series of tubes derived from this animal is as follows: The lung showed a good growth, the uterus quite abundant growth, the kidney less growth than either of the two mentioned, the placenta a doubtful growth, the liver and spleen no growth. An attempt was made to cultivate the bacilli in bouillon to which a bit of sterile lung had been added, but without success. Transplantations conducted as in the previous instance gave a progressively decreasing development upon the various organs during from two to three generations, after which no further growth could be obtained. It was impossible to obtain even a second generation upon the placenta.

Although we had now succeeded in cultivating the bacillus through two or three generations, the growth obtained upon the various organs mentioned was never abundant, and the limited development of the last transplantation was not regarded as offering favourable opportunities for the further experimental study of the cultivated organisms. It is therefore of interest to note that upon the fœtus of the rabbit transplantations from one of these objects to another was possible through a much larger series than in the previous cases. The method of obtaining the fœtuses was similar to that employed for obtaining the sterile organs. Pregnant female rabbits were employed, and care was exercised to obtain for this purpose such as had been impregnated probably ten days to two weeks before. The fœtuses which were most favourable for our purposes averaged from 5 millimetres to 1 centimetre in length. The exposed uterus was incised with sterilized instruments, the membranes separated, and then with a new set of sterile instruments the fœtuses were excised and introduced into recently sterilized test tubes. After a little practice this operation could be executed so expeditiously that only in rare instances was a tube found to have been contaminated. The sterile objects were then inoculated in the usual way with material derived from the experimental cases, and now for the first time an abundant growth was obtained. After twenty-four hours in the thermostat the tissues had become darker in colour, a slight scum seemed to overspread the surface, and some discoloration could be observed in the amniotic fluid in which the fœtus lay. Cover slips showed many bacilli of the length of the original forms, but differing somewhat in their staining properties. The organism was cultivated through a series of six of these objects, which represented the number of fœtuses obtained from one animal. There was no perceptible difference in the growth upon the sixth as compared with that upon the first. From the sixth generation intrapleural inoculations were made with the result of causing the death of the animal in three days, with lesions which were in all respects typical. This series of experiments was repeated at a later date with essentially the same results. It should be mentioned that the organisms which had ceased to

develop in the second or third transplantation upon the lung and the uterus, when transferred from these to the fœtus grew quite abundantly.

It will be recalled that among the early attempts which were made to cultivate the organism use was made of the heated and the unheated blood serum of the rabbit and of the coagulum from which the serum had separated, but with entirely negative results. After the successful experiments just mentioned it was decided to see whether or not a culture medium prepared from the organs of the rabbit upon which the bacilli grew could be used for their cultivation. With this end in view, a large pregnant female rabbit was killed by a sharp blow on the back of the neck. The abdominal and thoracic cavities were opened in the manner employed for removing the sterile organs. The heart and lungs, the kidneys, and the much-enlarged uterus, which contained eight well-developed fœtuses and placentæ, were removed and carefully hashed. An equal volume of sterile water was added and they were permitted to macerate in the ice chamber for twenty-four hours. The expressed fluid was passed through a Chamberland filter. The resulting straw-coloured, clear, and sterile fluid was introduced into test tubes to one part of which liquefied two-per-cent agar-agar at 40° C. was added. These tubes were permitted to harden in a slanting position. The remainder of the fluid was kept as such. Both the solid and the fluid media were inoculated from the local exudates of experimental animals and kept under aerobic and anaerobic conditions, but in no instance was the slightest evidence obtained of development upon either.

This experiment was repeated subsequently with the difference that the infusion was made entirely from pig's fœtuses, which measured each about 2 centimetres in length, but again with negative results. It may be mentioned, in passing, that as culture media for growing ordinary and, indeed, some unusual micro-organisms, both of these preparations answered exceedingly well. For example, the ordinary bacteria grew abundantly upon them, and the particular medium prepared from the pig's fœtuses proved to be suitable for the

growth of the gonococcus. We shall take occasion later to draw attention to the apparent significance of this observation as bearing upon the influence of certain constituents of tissues in determining the ability to develop, and the extent of development of certain micro-organisms.

The Organism as obtained from Cultures.—The variations in size and staining which have been described in connection with the bacilli obtained from various sources in the body of infected animals were again met with in the cultures. In the earliest cultures, as, for example, upon the kidney, lung, and uterus, there was a preponderance of the smaller forms. Among these some were as small as the least of those present in the experimental animals, although the greater number corresponded to two, three, or four of the dotted segments of the original bacilli. The long, threadlike forms did not, as a rule, reach the extreme length of the originals, and a tendency to the appearance of cross divisions was noted which separated some of these into pseudo-chains. The thickness also varied, the average breadth in the cultivated forms being somewhat less than in those obtained directly from the animal. The ends of the bacilli were rounded, and the great majority of both long and short forms stained solidly. Now and again slightly thicker ones were met with, which reminded one of the thick, solidly-staining rods sometimes seen in exudates, and these not infrequently were knotted and apparently undergoing involution. In the first inoculations from the animal the transplanted bacilli could usually be distinguished from the newly developed ones. The former retained their original forms, but they stained very slightly indeed, and were apparently undergoing degeneration. More than once was there observed an outgrowth from the original bacilli, which in the older parts were undergoing degeneration, while the growing end stained brightly and sharply. The degenerated end of such specimens could at times be seen to have the regular and characteristic dotted appearance, while the growing end stained equally and solidly throughout. The bacilli tended to grow in clumps, the longer forms overlying each other at various angles, the shorter ones appearing with-

out any striking regularity, at other times large and small forms were often intermingled in the growing masses (Plate IX, Fig. 2).

It was remarkable to what extent the nuclei of the tissue cells stained sharply and well, although the tissues had been removed from the body for many days, and now were overgrown by the organisms. A condition which might be termed pseudo-phagocytosis was occasionally observed in connection with these cells. The protoplasm, and more rarely the nuclei, were invaded by the bacilli, which sometimes were closely crowded within the protoplasmic substance of the cells, and again seemed to lie in vacuoles in the nuclei. The latter structures in the affected cells stained quite brightly, while the staining of the protoplasm was not to be distinguished from that of the uninvaded cells. It is safe to say that such an appearance observed in cells derived directly from animals would be taken to indicate phagocytic inclusions. It should be men tioned, however, that the long forms were not found inclosed in the cells.

Variation in Morphology produced by Inoculation.—It is apparent from the foregoing description that there is a distinct change in the general morphology of this organism in cultures compared with the appearances observed in exudates. While in the latter solidly staining forms are unusual, in the former they are not alone the most common, but after the second or third generation the only forms to be found. This peculiar, although not altogether new, observation of modification in form and staining properties in the cultivated organism compared with the more parasitic forms might be taken to indicate contamination at the time of the first transplantation, and, finally, a complete replacement of the original by the extraneous forms. What renders this improbable, and indeed quite inconceivable, is, in the first place, the fact that the cultures obtained from various sources and at different times all behaved in the same manner; and, secondly, that the inoculation into the pleural cavity of a rabbit of the organisms derived from cultures which had the morphology described in connection with these, set up a pathological process which was indistinguishable from the origi-

nal cases, and, what is of greater importance, the micro-organisms contained in the local inflammatory process again showed the typical irregular staining of the original bacilli.

Vitality upon Cultures.—An experiment was made to determine the duration of survival of the organisms upon sterile organs. On April 7th transplantations were made to the uterus and the lung. After twenty-four hours in the thermostat, at which time a fair growth had taken place, the tubes were removed, covered with rubber caps, and placed in a cupboard in diffuse daylight. They were removed on April 26th, and were found to have dried out to some extent. Cover slips showed a small number of organisms in these cultures. Transplantations were made upon the uterus, lung, and fœtus without obtaining any growth. It would therefore appear that the bacilli were incapable of surviving upon these media as long as eighteen days. From our previous experiments it is probable that the vitality is considerably less than this experiment would indicate.

The Histology of the Affected Organs.

The Lungs.—It has been pointed out that the pathological changes occurring in the pleura and the lungs of the spontaneous case do not differ essentially from those present in the experimental cases of pleural inoculation. It will be recalled that the pleura in these instances was covered with a shaggy false membrane, and that the affected cavities contained an increased amount of red fluid. The lungs presented, in some parts, appearances indicating that they were consolidated, while elsewhere they appeared to be collapsed, and in still other places to be insufflated. The bronchi contained, at times, pluglike masses which occupied their lumina, and which often extended into and quite filled the larger branches.

We shall take up separately the various structures affected. The exudate upon the pleura consists of large numbers of cells, the principal ones being polymorpho-nuclear leucocytes, although among these there are not a few which present larger vesicular nuclei. The amount of fibrin present within this exudate is surprisingly small,

although it varied somewhat, and was least abundant in the spontaneous case. The shaggy character of the membrane is found to be due to the large numbers of closely interwoven bacilli themselves, which imprison the inflammatory cells in their meshes. It is indeed difficult to give in a simple description an adequate idea of the enormous numbers of the latter which are contained in this false membrane. The pseudo-membrane is placed directly upon the serous covering of the pleura, and not infrequently the covering endothelial cells of the latter can be made out beneath the exudate. Indeed, they give the impression of having undergone proliferation, which fact may account for the relatively large numbers of cells with vesicular nuclei contained within the exudate.

The inflammatory process extended directly through the pleura into the substance of the lung. The superficial layer of lung alveoli, to a depth of two or three rows, is quite if not completely filled with a material resembling that present upon the pleura. Here, again, one is struck with the large numbers of bacteria present; and the manner of their growth through the pleural membrane can be readily determined. There is a direct extension from the surface into the most superficially placed of the alveoli, the bacilli occupying parallel rows and growing at right angles to the surface of the lung. They accommodate themselves accurately to the size of the invaded alveoli, often filling entirely by their growth the superficial layer, leaving practically no room for the exudative cells, which in these situations occupy the alveolar walls. Although the shapes of the bacterial masses here present appear to have been determined by the forms of the alveoli, it is interesting to note that the growth upon the surface of the pleura, which is in direct connection with the organisms in the alveoli beneath, presents much the same grouping, and strikingly suggests sheaves of wheat. Deeper in the substance of the lung the alveoli contain fewer bacilli, although a not inconsiderable number, and more of the inflammatory products. Where the bacilli and exudative cells are less abundant, fibrin appears more frequently in the inflamed tissue.

The affection of the bronchi is found to be far greater than was

evident at the autopsies. In no case was such affection absent, and frequently the bronchitis is a striking feature of the process in the lungs. Both large and small bronchi are affected, sometimes alone, but oftener with the involvement of the adjacent lung substance. The affected bronchi are, as a rule, completely plugged by a mixture of cells and bacteria. The predominating cells are again the polymorpho-nuclear leucocytes; and the bacteria varied in numbers in different situations within the bronchi. They are accumulated in greatest numbers directly in contact with the mucous membrane, at which situation they are disposed with great regularity. In the more central portions of the exudate they are fewer in number and more irregularly distributed. The grouping in connection with the mucous membrane is the same as that observed upon the surface of the pleura. The bacilli are disposed in parallel lines, which appear to start from an expanded base at the mucous membrane and to meet in a tolerably sharp point a short distance within the lumen of the tube, thus producing a series of conical elevations. The epithelium of the mucous membrane may be preserved, although it is always infiltrated with leucocytes. Now and then it has disappeared, and small defects exist in the mucous membrane; more rarely separated epithelial cells are found in the interior of the bronchi among the inflammatory elements and bacilli. The infiltration of the bronchi extends into the submucous, the muscular, and the adventitial coats, and thence, in certain instances, into the surrounding lung substance. The infiltration is accompanied with no small amount of cell destruction, as is shown by the nuclear detritus present in these situations. The size of the consolidated areas in the lung varies very much, and only rarely reaches the extent of the larger part of a lobe. In the case of a "lobar" consolidation there is a great want of uniformity in the appearance presented by the affected tissue. Some parts of it are firm and others are more loose in texture, presenting the picture of a number of smaller lobular foci of solidification, which have been more or less closely united by an intervening, loose inflammatory exudate. This appearance accords well with the involvement of the bronchi, which is greatest in the

areas of firm hepatization, and from which the lung parenchyma seems to have been invaded. The amount of cell destruction in the firmly-consolidated portions is often very great, and under the microscope may present an appearance much resembling the caseation of a tuberculous pneumonia. The bacteria are present along with the infiltration, and, indeed, appearances frequently suggest a reverse order of growth to that described; that is, from the affected lung tissue in which a relatively few bacilli are present the bronchi have been invaded secondarily, the rich development of the organisms occurring where they have free access to air—namely, next to the mucous membrane. The invasion of the bronchi in certain of the experimental cases could be observed to extend and ramify with the subdivisions of the larger bronchus. This has reference to the smaller tubes, which soon became lost in the lung parenchyma. Pictures showing the bronchioles, atria, and air cells which contained inflammatory products were seen. In the terminal bronchus the lining epithelium, now somewhat flattened, was not infrequently still present, whereas the exudate in the other structures invaded both the lumina and the alveolar walls, and thus obliterated the lining epithelium. In one instance we observed a continuous growth of the organisms, which extended through the ultimate structures just mentioned and gave rise, by the twisted character of its rounded contours, to an appearance suggesting the ramifications of a thick, corded rope. About some of the areas of broncho-pneumonia small foci of lung collapse occur. All of the structures about the bronchi, as well as the parenchyma of the lung itself, may be invaded with bacteria. It has already been mentioned that the several coats of the smaller bronchi may be the seat of the bacterial development, and in the larger bronchi neither the perichondrium nor the cartilage is spared, although the former is much more affected than the latter. The bacilli rather than the inflammatory cells invade the cartilage, and they are found both in the superficial and in the deeper layers between the cells as single bacilli or as small groups, occupying, apparently, the lymph spaces, and never, so far as was observed, actually invading the cells themselves.

The blood vessels in the areas of affected lung show interesting changes. A continuous extension of the inflammatory process from the lung substance, through the various coats of the vessels, including the internal coat, may take place, in which case large numbers of fragmented nuclei are present, both in the intima and in the lumina of the vessels. Such affected vessels contain thrombi of fibrin and leucocytes, which either partially or completely occlude their lumina. This inflammation of the vessels occurs in very large branches of both the pulmonary artery and the pulmonary vein. Another condition of really greater interest occurs very extensively in the spontaneous case, and not infrequently in the experimental cases. The vessels in the regions of the inflamed lung tissues show an increase of cells in the internal coat, which in the larger vessels distinctly narrow their lumina, while by the same process the smaller branches may be completely obliterated. The proliferated cells are for the most part large, possess vesicular nuclei, and resemble in size, staining properties, and relation of nucleus to protoplasm, the large mononuclear elements found normally in the blood. Among these a few definite polymorpho-nuclear leucocytes and an occasional small cell with deeply staining, round nucleus exist. An endothelial covering could often be made out, at least in the medium and larger-sized vessels, and the proliferated cells be followed to the internal elastic lamella, where they usually stopped. In not every case could a layer of flattened endothelial cells be found to cover the new cells, and the latter then might project directly into the lumina of the vessels. In very small vessels the flattening of the most superficial layer of cells is, as a rule, not present at all. Among the polymorpho-nuclear leucocytes within the blood of these vessels a large proportion of large mononuclear cells occur. At times these cells, apparently free in the current, communicate with the new cells in the intima, and, although we do not consider the evidence as sufficient, yet the possibility of derivation from this source of the increased mononuclear elements is too great to be overlooked.

The multiplication of the cells in the intima is by indirect cell division. The number of karyokinetic figures met with is surpris-

ingly large, and the figures are rarely seen in the depth of the new cells next the internal elastic lamella. From this it follows that the division takes place in the superficial rather than in the deeper cells. Indeed, the dividing cells are often the ones immediately next the lumen of the vessel, and in character resemble the vascular endothelium in the appearance of their nuclei only. We can not see why they may not have been derived from the endothelial cells into which they could readily be converted, in appearance at least, by simply becoming flattened. It is of still greater interest to find among the free mononuclear and polymorpho-nuclear elements in the blood current similar cells to those in the intima which are undergoing mitosis. The proliferated cells now and then arranged themselves in parallel lines at right angles to the lumina of the vessels, these lines being separated from one another by pale, apparently structureless fibrils without nuclei. Sections of the vessel which show the entire circumference indicate that the growth affects the vessel wall in its whole extent. That this process is obliterative in its tendencies, the study of vessels of various size leaves no doubt. It can only be surmised that in the end actual fibrillated connective tissue might result, and the final appearance be that of the commonly observed endarteritis and endophlebitis obliterans. The vessels which show changes in the internal coat are in the neighbourhood of the affected lung tissue; they are not, however, the seat of active changes in their other coats. So far as can be determined, they are not the seat of actual bacterial invasion.

The lymphatics of the lung, particularly those which run in the interlobular tissue, are greatly dilated, contain increased numbers of leucocytes, and not uncommonly fibrinous thrombi.

The bacilli are present in considerable numbers in all of the inflamed tissues, not excepting the walls and the lumina of the blood vessels, in which acute inflammatory changes (peri-arteritis, mesarteritis, endarteritis, etc.) associated with cell destruction were observed.

The staining agents which were employed in the study of the lung, as well as the tissues subsequently to be described, were hæmatoxylin and eosin and carmine for the tissue elements, fuchsin

and gentian violet for the bacteria, and Weigert's fibrin stain for the demonstration of fibrin. The most satisfactory results for demonstrating the bacteria were obtained with the methods which I had previously recommended for the staining of the typhoid bacillus in tissues.* It is as follows: Stain for a few minutes in Stirling's gentian violet (gentian violet, 5 grammes; alcohol, 10 cubic centimetres; aniline oil, 2 cubic centimetres; water, 88 cubic centimetres; filter), or one-per-cent carbolic fuchsin; place in 1 to 1,000 acetic-acid solution for some minutes, dehydrate and partially decolourize in ninety-five-per-cent alcohol, transfer to the slide, blot, and then add oil of cloves to clear and differentiate. Change the oil several times until the desired differentiation is obtained.

By the use of either of these stains, but particularly of the violet, the bacilli stain sharply and in many instances show the irregular appearances which constitute one of their characteristic features.

The Heart.—In this connection it will be necessary to treat of the lesions as they affect, first, the pericardium, and, second, the muscular substance.

Although some doubt was expressed as to whether in the case of the lung the bronchial infection preceded or followed the disease of the lung parenchyma, in the heart the two places of localization of the infectious material and the resulting inflammatory changes can be readily explained by the mode of distribution of the micro-organisms. For example, the pericardial infection occurred through direct extension from the inflamed pleura, whereas the foci situated in the depth of the muscle resulted from the vascular distribution of the bacilli. There is one exception to this statement—namely, in the case mentioned in which following the pleural inoculation there was in association with the pericarditis a single nodule or abscess present in the ventricular wall. That this was produced by direct extension from the pericardium can not be excluded, although it is equally impossible to say that the bacilli were not carried to the muscle either by the lymph or the blood channels.

* The *Johns Hopkins Hospital Reports*, vol. v, p. 366.

The pathological process present in the pericardium is strikingly similar to that upon the pleura. We have again a pseudo-membrane which varies in thickness, presents a shaggy appearance, and contains large numbers of bacilli. The same deception with reference to the fibrinous character of the exudate prevails here as in the case of the pleura, the large numbers of inflammatory cells being held together principally by the dense network of bacteria. A few small masses of fibrillated fibrin could, however, be seen in the exudate. The exuded cells are principally polymorpho-nuclear leucocytes, among which, however, not a few cells with single vesicular nuclei are to be found. The endothelial layer of the pericardium is generally not to be made out distinctly, because, perhaps, of the great infiltration; yet, in some places where the exudate is less thick, the endothelial cells can be seen to be preserved beneath the latter. The infiltration in those situations in which areolar and adipose tissue occurred penetrates a variable depth into these, and in those places in which there is no adipose layer between the serous membrane and the muscle an increase of nuclei has occurred between the muscle fibres. Some of the increased cells are certainly leucocytes; others appear to be proliferated, fixed tissue cells. Indeed, the latter seem to be derived, in part at least, from the pre-existing muscle nuclei. Deeper down in the substance of the heart, particularly about the larger vessels which enter its substance and around which the connective tissue is thicker than elsewhere, a moderate infiltration with small round cells and larger cells with vesicular nuclei exists. Thrombi occur in the lymphatics and veins of the pericardium, and many of the small veins are crowded with white blood-corpuscles. It should be mentioned that the sheaflike appearance observed in the massed bacilli in the lung and pleura was not to be made out in the pericardium. In this situation the organisms formed a fine and small meshed felted network in which many presented the characteristic dotted appearance.

The focal localizations of the bacilli within the muscular substance occur in all parts of the ventricular walls. They may be present just beneath the endocardium or just beneath the pericar-

dium. Where they occur beneath the pericardium an exudate of opaque and sticky character occurs over them, giving rise to localized foci of acute pericarditis. The abscesses occur singly as well as in small groups. As a rule, the single ones exceed in size a millet seed and can be distinguished from abscesses caused by the pus organisms by their firmness and the absence of tendency to liquefaction on the part of the tissues. Two appearances, apparently quite dissimilar, are obtained, according to whether the affected heart muscle is stained with ordinary nuclear dyes, such as hæmatoxylin, or with the anilines, which stain the bacilli rather than the tissues. For the study of the tissue elements the best results were obtained from the use of hæmatoxylin and eosin. The specimens stained in this way show in the affected areas large numbers of emigrated cells disposed between the muscle fibres, often in parallel or slightly angular lines. The outlines of the abscess are round, oval, or slightly more elongated. In the affected areas the actual number of muscle cells is diminished, at least so far as the demonstration of their presence is concerned. From the thinned and compressed appearance of the muscle cells it would appear as if there had been an actual loss in numbers; still, where the infiltration is so great it may be that there is an obscuring of the fibres and not an actual disappearance. The atrophied and compressed fibres described are modified in their staining properties. They present a homogeneous and hyaline appearance, have lost their striation, and are devoid of nuclei. They are evidently necrotic.

This change in the muscle fibres is not limited to the immediate contents of the abscesses, but extends a variable distance from the periphery, and may affect groups of fibres quite removed. The affected fibres not directly in the abscess contents may still possess colourable nuclei, although these are more or less modified. They are smaller than the normal nuclei and take a very deep and opaque hæmatoxylin stain. It is more exceptional to find in the muscle fibres within the abscess the retention of their nuclei. Fragmented nuclei are present in relatively small numbers, and there would appear to be but little change in the fixed cells about the affected

areas. The gentian-violet stain shows a most extraordinary development of the bacilli in the abscesses. They surround the individual muscle fibres, which are pale and colourless, and are identified by their form and, occasionally, where these are retained, by their nuclei.

The bacilli are, however, not limited to the capillaries, but, corresponding to the points of atrophied or even deficient muscle fibres, they form an interlacing, close-meshed network within the tissue. Their thickest development is not in the centre of the abscess but is at the periphery, the network in the centre being much more delicate than at the outer zone. The bacilli do not end abruptly at this outer zone, but may be followed in the form of small groups and even singly between the adjacent muscle fibres, and apparently here they are chiefly within vessels. It can not be stated that there is a growth directly into the muscle fibres, although appearances suggestive of this were observed. The aniline stain also brings out the fact, less noticeable in the tissue stained for the tissue elements alone, that there is a much more extensive destruction of cells, principally through a fragmentation of their nuclei, than was before evident. These fragmented and necrotic cells lie in the periphery of the abscess and occupy definite spaces between the muscle fibres, which may be capillaries or lymphatics. Intermingled with the cellular detritus there are many of the characteristic bacilli.

From the descriptions of the lesions in the pericardium as well as in the heart's substance, it will be noted that in the first place the changes are of the nature of an acute serositis associated with a moderate interstitial myocarditis, and that, as compared with staphylococcus abscesses, the abscess formation here is peculiar, owing to the absence of evidences of softening of the tissues and because of the localization of the bacilli in the periphery of the cellular infiltration rather than in its centre. The degenerations produced in the muscle cells themselves are very extensive, and vascular changes of a proliferative character, similar to, although less extensive than, those in the lungs, take place. Thrombi occur both in the vessels beneath the pericardium and within the muscular substance, but

undoubted examples of proliferation of the cells of the intima have not been found associated with pericardial affection alone.

The Brain.—The microscopical study of the cases of meningeal infection show that the lesions are not limited to the membranes, but that the brain substance suffers as well. Even in the earliest cases—for example, in the one in which death occurred twelve hours after inoculation—the lesions, both in the meninges and in the cerebral cortex, are well advanced.

Corresponding to the seat of inoculation there is a rich infiltration of the leptomeninges, which is in the form partly of tolerably circumscribed nodules of pus cells and partly of a diffuse infiltration with the same. The thickness of the exudate is less in the diffusely infiltrated parts. The adjacent brain substance is not the seat of abscess formation. On the other hand, for the distance of 1 millimetre, or thereabouts, the cerebral cortex is more than normally condensed, stains deeply in eosin, has suffered disappearance of tissue cells, and a moderate infiltration with polymorpho-nuclear leucocytes. Bacilli are in this case fairly abundant only, and are limited, so far as the sections indicate, to the meninges. Where the duration of life was greater, as in the case of the animal which survived a subdural inoculation for twenty-two hours, the lesions are more advanced. The meninges show similar but diffuse infiltration, whereas the underlying brain substance is greatly injured.

The superficial cerebral cortex, corresponding to the seat of inoculation and extending several millimetres to each side of it, is completely necrotic, devoid of well-preserved cells, and contains fragmented nuclei. In the midst of this necrotic material a small abscess was found. The inflammation extends directly into the lateral ventricle of the inoculated side, although the brain substance in its entire depth was not so greatly modified as that near the seat of inoculation. A continuous growth of bacilli, which in the deeper parts is very slight in amount, has penetrated into the ventricle. The choroid plexus is greatly infiltrated with pus cells, and these, mixed with red blood corpuscles, also occur in the ventricle where a great increase in the number of bacilli has taken place. The ependymal

epithelium appears intact. A surprising feature of this case was noted in the cortex just beneath the seat of inoculation, where a triangular gas cavity, 2·5 millimetres in diameter in its widest part, existed. The walls of this are composed of the condensed and necrotic brain substance in which a very slight invasion with leucocytes has occurred. The number of bacilli in the pia mater and in the adjacent brain substance is very great indeed, but the greatest accumulation occurs in the condensed tissue forming the walls of the gas cavity. Although fragmented nuclei are abundant in the necrotic brain substance, it is remarkable how few emigrated cells occur in this situation.

The brain abscesses produced by the intravenous mode of inoculation varied, as was stated, in the number as well as in the size of the individual abscesses. They differ also in appearance according to the manner in which the sections containing them are stained. Hæmatoxylin and eosin show the presence of considerable numbers of polymorpho-nuclear leucocytes in the affected areas. The smaller abscesses are sharply circumscribed by the surrounding brain substance, while the larger ones spread in a peculiar and interesting way and are less sharply limited. Those abscesses the contours of which are definite, are packed so closely with leucocytes that there appears to be an entire absence of intercellular material of any kind.

On the other hand, the larger and irregularly defined ones show smaller collections of leucocytes irregularly distributed throughout the abscesses and frequently well separated from one another by intervening, faintly fibrillated material, which by this stain could be seen to be composed of masses of bacteria. The larger accumulations of leucocytes occur in the centres, the smaller eccentrically, and the periphery frequently is entirely or quite devoid of them (Plate X, Figs. 3 and 4). The deeper-placed abscesses are separated at times by a very narrow strip of tissue from the interior of the ventricles, and in those cases in which the abscesses approach the ventricle an acute ependymitis always exists. The brain tissue about the abscesses is greatly altered. Where the abscesses are spreading

in character there is extensive necrosis of cells with fragmentation of nuclei, amounting at times to complete destruction of the adjacent brain substance.

The further removed from the abscess the affected tissue lay, the less intense the effects, until only increased granulation of the protoplasm of the cells distinguish the injured from the normally staining ones. Glia cells and ganglion cells both suffered, although the protoplasmic changes mentioned were observed in connection with the ganglion cells. The blood vessels, particularly the capillaries and smaller veins and arteries, show varying degrees of involvement. Those most affected are infiltrated with cells, many of which are fragmented, the resulting detritus often following the perivascular lymph spaces and appearing also within the lumina of the vessels, which usually are thrombosed. The larger vessels, and the ones at the same time least affected, show on a small scale the increase of intimal cells similar to that described in the lung.

The relation of the bacilli to the circumscribed abscesses is that of a rather indiscriminate mingling of organisms and cells. Between the closely packed emigrated cells aniline stains show a considerable number of typical rods. On the other hand, in those abscesses which are less circumscribed the bacilli form only tolerably dense masses in the centre, the greatest growth being in the periphery, where the organisms invade the tissues in parallel lines, forming a continuous envelope which surrounds the accumulated leucocytes (Plate X, Fig. 3). Higher powers of the microscope show a finer invasion still, extending into the adjacent brain substance for a considerable distance beyond the continuous envelope mentioned. Single bacilli also push their way into the cortical substance between the nerve cells, often surround these, and apparently lie within their perivascular spaces (Plate X, Fig. 4). The tissue in which the large masses of organisms are contained shows complete absence of staining, whereas that in which the single forms occur exhibits only the protoplasmic alterations of the nerve cells already described. The micro-organisms appear capable of invading the capillary walls and of gaining entrance directly into the interior of the vessels, in which instance fragmenta-

tion of the nuclei of the endothelium results. The meninges do not, as a rule, suffer; the most discovered being a slight increase in the number of cells in the leptomeninges just over the superficially placed miliary abscesses.

This series of cases illustrates the variety of lesions which this organism is capable of producing when it finds its way into the central nervous system. We have as its earliest effect the acute inflammatory affections associated with a moderate increase of the number of organisms introduced, and other lesions which, from their nature and from the absence of the bacilli within them, would appear to be the result not of the bacterial invasion as such, but of the action of some toxic material which they produce. The changes observed in the cases of rapid death succeeding subdural inoculation are of this nature. The effects of the rapid development of the bacilli would appear to be rather of an actual necrotizing nature, such as we see in the second case of meningeal inoculation and in those cases in which there is a diffuse invasion of the brain by the organisms. In this necrotic tissue there may be an entire absence of stainable nuclei, and the intensity of the action of the poison is illustrated not only by this fact, but also by the nearly complete absence of emigrated cells. An interesting phenomenon is the ability of the organism to produce gas within the cerebral tissues, as is shown by the cavity described in connection with one of the meningeal cases. This case is further remarkable from the fact of the growth of the bacilli from the surface of the brain directly through its substance into the ventricle. The disposition of the bacilli in some of the abscesses is worthy of special note. If one of these be compared with experimental staphylococcus abscesses, the difference in the localization of the organisms becomes at once apparent. In the latter there is usually a central mass of bacteria about which the emigrated cells accumulate, and only smaller groups of organisms occur throughout the abscess contents intermingled with the pus cells. The peculiarity of the distribution which we have described is found in the extensive peripheral invasion of bacilli away from the centrally accumulated masses of pus cells next to which they are not present in

any considerable numbers. Attention has already been drawn to the striking regularity of growth exhibited by this bacillus. It is not better shown in any situation than in the central nervous system, where it forms palisadelike inclosures of the emigrated cells.

The Liver.—The histological examination of the liver in those cases in which abscesses were present in this viscus indicates that the lesions are of several distinct varieties. The structure of the abscesses themselves is modified only by the difference of the tissue in which they are located as compared with those already described in other places. There is a rich development of bacteria within the affected area, usually more marked in the periphery than in the centre. The bacteria occupy especially the capillaries, although where the liver tissue is much damaged, as is likely to be the case near the centre of the focus, they grow indiscriminately through the tissues. A rich invasion with polymorpho-nuclear leucocytes, which are distributed quite uniformly in the affected foci, has taken place. In most instances there has been great necrosis of the involved liver tissue, which is shown by the absence of definite staining and the great numbers of fragmented nuclei in these situations. Indeed, under the low power, the appearance in the hæmatoxylin- and eosin-stained preparations is very suggestive of caseation. Similar appearances, it may be recalled, have been described in connection with certain broncho-pneumonic patches and brain lesions.

The larger blood vessels near the abscesses are thrombosed usually with fibrinous coagula, but now and then a large branch of the portal vein, or more rarely of the hepatic vein, has been invaded by the bacteria, which may completely fill its lumen. Single abscesses exist, although there is greater tendency to the occurrence of groups, without, however, actual confluence taking place. On the other hand, the abscesses are frequently united with one another by irregularly-sized areas of necrosed liver tissue.

The necrosis of the liver cells is very striking, and consists of the conversion of larger and smaller areas of the liver substance into an irregular staining and appearing tissue. The cell protoplasm is

greatly modified; it has become homogeneous and often contains large vacuoles. The arrangement of the cells in rows is much disturbed; in many the cells have lost their connection with each other; the capillaries have collapsed and may be entirely indistinguishable. Again, in places the liver cells have disintegrated and even disappeared, leaving an irregular meshwork of hyaline appearance which may represent the modified remains of capillary walls, although nuclei are not to be made out in them. Some of the liver cells with altered protoplasm retain their nuclei, but these are shrivelled, contracted, and irregular in outline. Others contain fragments of nuclei, and into still others several polymorpho-nuclear leucocytes have wandered. Occasionally a hæmorrhage has taken place into such an altered area of tissue. The whole, or nearly the whole, of a lobule may be included in the necrosis, which, from the number of leucocytes present, would appear to be strongly chemotactic. The liver cells generally are the seat of fatty changes. No bacilli were demonstrable in these necrotic foci. Similar forms of hyaline necrosis of liver cells not infrequently occur at considerable distances from the abscess formation, and indeed they have been frequently found in the liver both of rabbits and of guinea-pigs in which pleural inoculations had been made and in which there was an entire absence of localization of bacilli within the liver substance.

Still another form of necrosis was occasionally met with in portions of the liver more or less removed from the abscesses. In such places the capillaries are entirely occluded by a rich and thick growth of the bacilli, which often assume a convoluted appearance, and which, in properly stained specimens, give to these situations the appearance suggestive of a liver in which the blood vessels have been injected. The rows of liver cells between these capillaries refuse to stain at all excepting to a slight extent in eosin. The nuclei of the cells are usually so pale as hardly to be distinguished, and are frequently entirely wanting. A very few nuclear fragments exist among these cells. The appearance here suggested is of an anæmic (coagulation) necrosis. The capillary blood vessels immediately surrounding the abscesses are greatly dilated and turgidly filled with

blood, the rows of liver cells being correspondingly compressed and narrowed.

The effect of the larger abscesses upon the surrounding liver tissue is to compress greatly the latter, causing the liver cells to become elongated and spindle-shaped. There is no suggestion whatever of fibrous encapsulation. The exudate upon the capsule of the liver, which was always present where the abscesses came to the surface, consisted of polymorpho-nuclear leucocytes, bacilli in great numbers and fibrin. The inflammation from within could be observed to pass directly through the capsule of the liver and thus to infect the peritoneal surface. In some cases an accumulation of inflammatory cells took place between the liver parenchyma and the capsule, and subsequently it seemed to penetrate the latter. Where this occurs the capsule itself is densely infiltrated with cells, and its endothelial covering either entirely destroyed or so obscured as not to be made out.

It is worthy of note that the intravenous inoculation of the bacilli did not always result in the production of definite abscesses in the liver, and that in those cases in which they did appear there was evidence of previous disease of this organ. For example, either definite psorosperm nodules existed, or a well-marked cirrhosis, which might have been taken to have resulted from the presence of parasites or from the action of other injurious substances, the presence of which within the sections could not certainly be demonstrated.

The lesions in the liver may be considered as representing three distinct types: First, abscess formation which is attributable to the localization of the bacilli within the tissues, the destruction of the latter, and the emigration of large numbers of leucocytes from the blood; second, more diffuse necrotic lesions, due not to the direct action of the bacilli, but produced through the presence of the toxic products of their growth; third, a form of anæmic necrosis caused by extensive obstructions of the blood capillaries by an abundant growth of bacilli in the affected part.

The Peritonæum.—The character of the diffuse peritoneal exudate has already been described in connection with the liver, and it will

be recalled that it consists of a mixture of emigrated white blood-corpuscles, bacilli and a small amount of fibrin. Where the inoculations were made directly into the peritoneal cavity and the incubation period was greater, a diffuse and moist peritonitis did not, as a rule, occur, but small nodules of grayish or yellowish colour were found upon the parietal and visceral coats. Sections carried through these showed a somewhat different structure from those described. The exudate in these cases is firmer than in the more acute ones, and under the microscope it is found to consist of cells and bacilli, with the complete or almost entire absence of fibrin. There is not so great a preponderance of leucocytes present, many of the cells having the characters of the small round elements and of epithelioid cells. The thickness of the exudate reaches 2 or 3 millimetres, and it is placed directly upon the serous coat, the endothelial layer of which is demonstrable in places beneath it.

Amid the inflammatory elements there are the greatest numbers of bacilli. These form a felted network which in hæmatoxylin-stained specimens might be mistaken for finely fibrillated fibrin. More circumscribed accumulations of cells occur within the general exudate, and have the size and appearance of small discrete abscesses. In the centres of some of these, large masses of bacilli, showing the peculiar sheaflike appearance already described, are to be observed. The leucocytes are massed about these, being fewer in the centre than in the periphery. The muscle underlying the serosa is infiltrated to a small extent with polymorpho-nuclear cells. On the other hand, there has been an increase in the nuclei of the sarcolemma. The blood vessels contain an increased number of leucocytes, and the arteries show upon the side nearest the inflamed peritonæum a proliferation of intimal cells similar to, although less extensive than, those described in the lung. Small thrombi are met with in the veins, the walls of which are free from thickening, such as is present in the adjacent arteries.

The Subcutaneous Tissues and Muscles.—The inflammation of the subcutaneous tissues does not in any instance reach the skin surface. The corium itself is very slightly affected as compared with the

subcutaneous connective tissue. The lesions here are in the very acute cases extreme, and consist of œdema and an extensive destruction of cell nuclei with a moderate invasion only of polymorpho-nuclear leucocytes. In the deeper layers of the subcutaneous tissue the destruction of cells is less and the emigration greater. The adipose tissue is peculiarly affected. It appears as if the fat had been liberated from the cells containing it and had aggregated in tissue spaces lined with endothelium, the intervening connective tissue of which is densely infiltrated with cells. In the more chronic cases there is less œdema and less fragmentation of cell nuclei, and indeed less cell infiltration. Just before the superficial muscle is encountered the emigrated and fragmented cells are gathered in very great numbers, presenting the appearance of a definite abscess. The walls of larger veins are completely necrotized and the veins themselves are thrombosed. The arteries may show little change, or fragmentation of a few muscle cells and many intimal cells; they may not, however, be occluded. The affection in the arteries is greatest on the side next to the inflamed part.

The muscle shows varying degrees of affection. Immediately within the infiltrated area it is hyaline and has lost its striation as well as its nuclei. The intermuscular septa are densely infiltrated with polymorpho-nuclear cells which frequently invade the muscle itself. In long sections of the muscle the affected fibres may be seen to be undergoing a gradual metamorphosis with the production of a material resembling hyaline, which exists at times in the form of drops. This muscle shows a peculiar reaction to hæmatoxylin, which stains in particles and lines, suggesting in a measure the striations of the original fibres. The greatest accumulation of polymorpho-nuclear cells takes place beneath the muscle in the deeper fascia, where they are present in such great numbers as to obscure the remainder of the tissue.

The relation of the bacilli to the affected subcutaneous tissue and muscle is as follows: The superficial œdematous connective tissue in which such great numbers of fragmented nuclei occur is entirely free of bacilli. The latter do, however, occur in the deeper and more in-

filtrated parts, where they dispose themselves in wavy, parallel lines, corresponding with the lines of the curly fibrous tissue with which they might readily be confounded. They evidently lie in the spaces between the fibres. The numbers here are enormous, but their greatest development is reached between the affected muscle fibres, which they entirely surround and inclose, and into which they may, in rare instances, be seen to penetrate. They are present again, intermingled in an indefinite way with the pus cells of the deeper abscess formation. Fibrin was not made out in either the subcutaneous tissues, the muscle, or the deeper cellular accumulations.

The Uterus.—In the case of spontaneous death the extent of affection in this organ varies with the local conditions. In those situations in which decidua and placenta are entirely wanting the invasion of bacilli is far less than elsewhere. The surface epithelium is in these situations usually intact, and a thin exudate, composed chiefly of cells, lies upon the surface. The glands are not particularly dilated, but the epithelium which lines them is for the most part desquamated and even degenerated. It very rarely happens that a gland has retained the epithelium in contact with its walls. Among the few preserved cells within the mass inside the gland there are many fragmented nuclei, and occasionally a polymorpho-nuclear cell. The most striking feature is the relation of the bacilli to these structures. The growth upon the surface is fairly abundant and intermingled with the cellular exudate. On the other hand, all the glands have been invaded by a rich bacillary growth, which has interposed itself between the masses of degenerated epithelium and the gland walls. An outline of each gland can be obtained from the growth within it, and here again we notice a more marked tendency still to grow in parallel lines, producing sheaflike masses. (Plate X, Fig. 5). The underlying tissues of the mucosa and of the muscle are much less affected, and show at most a slight accumulation of fragmented cells and a few bacilli in their substance.

A different picture is presented in those situations in which the decidua still exists within the mucous membrane. Sections were carried through various segments in which the thickness of mucous

membrane indicated that the decidua was present, and it may be noted that in none of these did the decidua extend throughout the entire circumference of the mucous membrane. It usually occupied about two thirds of the circumference, leaving the remaining one third of mucous membrane provided with quite normal-appearing glands, although the superficial epithelium over the latter was rarely to be made out. A distinct separation of the decidua into a compact and spongy layer was, as a rule, impossible; occasionally a layer of closely packed cells with prominent and often multiple nuclei could be seen upon the outer surface of the membrane. For the most part, the outermost layer consists of a wide-meshed vascular tissue in which a few remnants of gland structures can be discerned. The walls of the venous sinuses and arteries are composed of quite large cells, with vesicular nuclei, which are separated by an attenuated, quite homogeneous, intracellular substance, and they are infiltrated with smaller cells, containing more deeply staining nuclei. In the deeper parts the glands are more numerous and better preserved, although they are often dilated. The tissue which separates the glands from one another presents in general the same characters as that described as existing between the blood vessels of the upper layer. The normal stroma of the mucous membrane is entirely absent in the situations of decidua formation, although it is present in the adjacent and unchanged mucosa. The blood vessels beneath the mucous membrane are enormously dilated, and the decidual vessels turgidly swollen and filled with blood. The relation of the microorganisms to these structures of the uterus is of the most intimate character. In the decidua the bacilli seem to have found most favourable opportunities for development, and they have spread out freely in the intervascular cellular tissue and penetrated throughout the depth of the transformed mucous membrane. The bacilli are not absent from the blood vessels, although they do not occur in them with great frequency; yet what appears to be a section of an arterial segment is quite filled with a bacillary mass. Wherever glands remain, there the largest single aggregations of the organisms are found. In their free spaces a return of the tendency to grow in

sheaflike masses is always apparent. Within the decidua itself the growth is more irregular, producing a felted network of organisms. In that portion of the mucosa in which actual decidua was absent the development of the bacilli is also very marked; and in these portions of the uterus the muscle shows greater changes, particularly of infiltration and cellular destruction, associated with a slight but certain invasion of the micro-organisms. Inflammatory cells, consisting chiefly of polymorpho-nuclear leucocytes, occur in considerable numbers in the affected decidua and mucosa, while they are present in increased numbers in the circulating blood within the vascular channels. Thrombi, composed of hyaline fibrin mixed occasionally with leucocytes, occupy many of the deeper vessels of the decidua, the mucous membrane, and the muscle.

The dilated segments which are described in the earlier part of this paper, and which, it is believed, owe their peculiar appearances to the presence of a gas, are now seen to be greatly attenuated, the thinnest portion being reduced almost to a line. The mucous membrane in the less affected parts show numerous glands, and in the most highly affected part the merest remnants of these structures. Bacilli are tolerably abundant throughout the mucous membrane. The points of greatest attenuation are opposite the attached end—that is, in the unattached portion of the uterus. The mucous membrane and underlying structures of the attached end are several times thicker than the greatly attenuated tissues of the unattached end. In the latter situation the muscular coat suffers an extension as great almost as the mucous membrane. Evidently the support afforded by the abdominal tissues of the attached side offered an obstacle to the dilating influence of the accumulated gas, which was exerted with greatest effect upon the free portions of the affected segments.

If we turn now to the experimental cases we shall find much the same condition as we have described in this one. The study of these cases has been limited to the uterus itself, and has not extended to the tissues of the fœtuses which remained. It is found that the decidua has essentially the same structure, occupies the same position in the mucous membrane as in the natural case, and

has afforded a similarly favourable opportunity for the development of the bacteria. In one class of cases (intravenous inoculation) these were brought to it by the circulating blood, notwithstanding which fact they are not found abundantly within the larger vessels. They are probably often in the capillaries, although they are very exceptionally found in definite veins or arteries. The outermost layer of the decidua frequently presents an extensive necrotic appearance, and the blood vessels are thrombosed in these portions, and also, although to a less extent, in the deeper layers. The superficial parts of the decidua contain but few glands, whereas the deeper parts contain many more, and these are usually dilated. There seems to be no special difference to be made out in the localizations here, as compared with those of the spontaneous case, although it may be remarked that in actual numbers the bacilli seemed to exceed those present in the first instance. The intervening portions of the uterus, between the segments which contain decidua, are much more nearly normal in appearance; and, as in the original case, these are parts of the mucous membrane which are not at all concerned in the pregnancy. Although the micro-organisms are present within the mucous membrane in these latter situations, they have not developed to the same extent as in the former, nor have they, apparently, produced the same damage. Still, it should be remarked that the gland epithelium itself has suffered desquamation and degeneration. The histological appearance presented by the dilated pouches in the experimental case in which gas was believed to be present in the uterus is similar to that described in the spontaneous case, and it is to be regarded as due to the same agency.

The instance of intrauterine inoculation affords the best example of the invasion of the various coats of the uterus. There are not alone great changes, similar to those already described, to be made out in the mucous membrane and decidual tissue, but the muscle of the uterus is œdematous and infiltrated both with large numbers of bacilli and with inflammatory elements. The inflammation extends to the serous surface, which is covered by a mixture of fibrin and cells, among which large numbers of bacilli occur. The intervillous spaces con-

tain great numbers of bacteria, and the surface of the mucous membrane is covered with a rich growth of the organisms, among which very few tissue or emigrated cells can be made out.

The increased susceptibility of the pregnant compared with the non-pregnant uterus to the localization, development, and injurious effects of this organism, is very conclusively illustrated by this series of cases, and indeed as much so by the study of the relation of the bacilli to the various portions of the affected uterus as by the observation of the differences in behaviour of the pregnant organ on the one hand and the non-pregnant on the other. From the pathological process observed in the affected structures produced by the intravenous and the intrauterine inoculations, and from their agreement with the observed conditions in the spontaneous case, it could be easily imagined that the presence of the bacilli in the uterine cavity might have brought about a premature labour in the first case, as it demonstrably did in the experimental ones. In none of the experimental cases of uterine infection were localized inflammations met with similar to those in the pleura and pericardium of the spontaneous case.

The Remaining Organs.—Very little need be added concerning these. With the exception of the liver, which in many cases showed focal areas of necrosis of the liver substance, focal lesions were not found in the viscera, in which no localization of the bacilli took place. The hepatic necroses, it must be assumed, were produced not by the bacilli themselves, but by the soluble products of their growth. However, until a certain and convenient method for the cultivation of the organisms is found, this point can not be entirely settled, for we know that the use of cover-slip preparations for determining the presence or absence of micro-organisms in tissues is not in itself conclusive. We have, however, abundant analogies tending to show that similar lesions can be produced by the products of bacteria, and it is therefore, perhaps, not assuming too much to consider that this one also is capable of manufacturing a toxic substance which, in the absence of organisms themselves, may be destructive to tissue elements situated at a distance from the seat of its formation.

Résumé and General Considerations.

The organism described in the foregoing pages must, we think, be regarded as belonging to the pleomorphic bacteria, although it may not be entirely clear precisely with which group it should be classed. Assuming that we have before us the entire series of its developmental phenomena—which, in the absence of a knowledge of more durable forms, or the conditions under which it may vegetate outside of the animal body, may well be questioned—it is possible to exclude it from certain groups of more highly organized bacteria. The complete absence of branching, both apparent and real, removes it from the group of cladothrix on the one hand and streptothrix on the other. With the leptothrix forms it has more in common; and from its tendency to grow into long and slender threads, and in cultures to appear in segmented filaments, it might, provisionally at least, be classed with this group. A further support for such a classification might be found in the presence of a sheathlike mantle, in which the chromatic particles are distributed (crenothrix), were it not for the fact that the nature of these particles hardly permits of their being regarded as independent bacterial cells; nor do they ever appear separately and independently of the sheath in preparations obtained directly from the animal body, while in the cultures no suggestion of a sheath is to be made out.

Notwithstanding the great variations in size, and the slighter differences in staining properties which were observed in the organism as obtained from the original source, and again noted in all subsequent cases, there can be no reasonable doubt that a single species existed in the infected animals as well as in the cultures. It would be, to say the least, remarkable if, in the various transplantations from animal to animal, and from these to culture, and then back again to animals, two distinct organisms had remained together in so nearly the same ratio as in this instance. It is no uncommon experience to have under observation mixtures of bacteria in cultures, but it usually happens that one or the other kind possesses the power of greater and more rapid growth, so that after a series of trans-

plantations the weaker or more feebly growing organism is overgrown, and, if not entirely destroyed, so greatly reduced in number that especially favourable conditions are required in order that it may again make its appearance. What happens in artificial cultures takes place more rapidly and certainly when the living animal body becomes the territory upon which the striving micro-organisms seek to implant themselves. We know, from the results of the earlier experiments with mixtures of bacteria carried out by Koch, that the animal body is capable of separating pathogenic species of bacteria from non-pathogenic, as well as different pathogenic forms from one another. It is therefore hardly to be conceived that two or more distinct species should have continued to flourish together side by side through so long a time, and under such varying conditions as were present in these experiments. On the other hand, it is no new fact in bacteriology to find a single species of bacteria under such manifold forms as in the present case. Indeed, it may, after all, be questioned whether the variations between the long and the short forms and the dotted and the solid rods are as great as between the extremes of proteus vulgaris.

The greatest changes observed were in the artificial cultures, for in the last removes the irregularity of staining quite disappeared, the bacilli appearing now as solid rods, many of which could be seen to have divided by transverse segmentation. Even if the evidence were not at hand that, as obtained directly from the animal body, a solid form is occasionally observed among the irregularly staining bacilli, and that the inoculations into rabbits of the artificial cultures containing solid forms cause a typical pathological process with a return of the characteristic markings in the micro-organisms, we have abundant observations upon the changes in the morphology of the common bacteria that may be produced by differences in the composition of the media upon which they are grown, and upon such of their physiological functions as the production of pigment, of acid or of alkali, of toxic substances and of spores.

The peculiar selection which this organism exhibited in respect to the substances upon which it was finally brought to develop out-

side the body of infected animals need excite no especial surprise if it is remembered that not a few widely distributed and quite commonplace bacteria—namely, the gonococcus, the micrococcus lanceolatus, and the streptococcus pyogenes (Marmorek)*—show at times similar preferences for certain media, and refuse entirely to grow upon the more ordinary artificial nutrient compounds. Moreover, as is well known, there are not a few bacteria which, while easily demonstrable in cover-slip preparations, have not been successfully cultivated at all. Whether the mouth bacteria, which are so refractory in this respect, might not be brought to regular development upon media that more nearly represent the fluids and tissues of the animal body than do our artificial compounds, may well be asked. Thus the behaviour of the more purely parasitic bacteria upon the ordinary culture media has made it quite unmistakable that under the most favourable circumstances we have in the latter only doubtful substitutes for the tissues themselves, a fact which these results confirm and extend. The further demonstration of differences in the individual organs in their behaviour toward the growth of this bacillus is also of interest in connection with the metabolism of these organs and the observed localizations of the micro-organism within the body.

The question of the effects exerted upon bacteria by the soluble constituents of the different tissues has recently been studied in Schottelius's laboratory, and several publications have already appeared upon this subject. The organs employed were the thyreoid gland (Kopp),† the pancreas (Kotlar),‡ the kidney (Henssen),# and the liver (Onufrowicz).‖ Culture media prepared from cold and filtered infusions of these organs affected the growth of bacteria in various ways. Some were restrained, others accelerated, and some suffered changes in their morphology. The last were not of a permanent character, a reverse change taking place upon retransplantation to the ordinary nutrient materials.

* *Annales de l'Institut Pasteur*, No. 7, 1895.

† *Centralblatt f. Bakteriologie u. Parasitenkunde*, Band xvii, 1895, p. 81.

‡ *Ibid.*, p. 145. # *Ibid.*, p. 401.

‖ *Die Lebernährböden und ihre Beziehung zum Wachsthum und Differenzierung der Bacterien.* Inaugural Dissertation, Freiburg, 1894.

The study of Henssen in particular seemed to indicate that the staphylococcus pyogenes aureus, which in rabbits often produces abscesses in the kidney, grows very slightly upon infusions of this organ. Bearing upon this point are our studies upon the relative values of the different solid organs, as culture media and their relation to the localization of the bacilli within the body. It was, however, not possible to make any comparison of the behaviour of the organs and of media prepared from them, as upon the latter no indication of growth was ever obtained. The two organs upon which a growth was never obtained were the liver and the spleen. The kidney permitted the transplanted bacilli to develop, yet the growth was slight in amount and more quickly refused further transplantation than that present upon the other organs. These facts are not altogether without significance when we compare them with the observed localizations of the bacilli within the organs. The kidney and spleen in no instance showed focal lesions produced by the bacilli themselves, and when such were found in the liver this organ was invariably the seat of previous disease. On the other hand, the growth upon the removed organs was more vigorous in the case of the pleura, the lung, the pericardium, and the uterus, and these were the situations in which the bacilli produced the greatest damage within the body. It is an attractive idea to consider that there may be a relation between the predilection shown by micro-organisms for certain viscera and certain chemical substances contained within these structures. According to this idea, the fœtus of the rabbit would contain these substances in larger quantities than the viscera, or in a more easily assimilable form. These hypothetical substances also would appear not to pass over into the fluid in which the organs are macerated. The foregoing is to be considered at most as a suggestion for which the only basis are the observed facts enumerated. It is true that a contrary mode of explaining the facts is not to be excluded—namely, that in some of the organs substances actually injurious and inhibiting in their nature may be contained, to which the variation in or entire absence of development is due. It is not possible to explain the distribution of the bacteria solely through

the peculiarities of the blood supply in the organs. To make this clear it need only be asked why the kidney, in which the arteries are terminal, should always escape, and the voluntary muscles sometimes be the seat of abscess development?

The probable nature of the deeply staining dots, globules, and cylinders within the bacilli is of interest. That they contain the main if not the entire chromatin material is already evident from the descriptions, as well as from the photographs and drawings. They present, however, certain peculiarities which are worth mentioning. The bacilli are easily stained in the basic anilines, gentian violet, fuchsin, and methylene blue, but least rapi lly in the last dye. In stained specimens which have been washed simply in water the threads stain fairly, although not quite uniformly; however, the deeper-stained dots can still be observed within the deeply tinted protoplasm. The use of a weak decolourizing agent (1 to 1,000 acetic acid) brings about a sharp differentiation between the chromatic particles and the matrix of protoplasm in which they lie. The use of stronger decolourizing solutions—as, for example, one-per-cent sulphuric acid, as is used in Neisser's method—causes instantaneous and complete decolourization, and attempts to obtain double staining in methylene blue and Bismarck brown by Ernst's method were entirely unsuccessful. On the other hand, immersion in moderately strong solutions of Bismarck brown for some minutes produced the effect obtained only with the use of bleaching agents after staining in fuchsin or gentian violet. The sharpness and distinctness with which the rods stained in hæmatoxylin were remarkable. This reagent stained the chromatic particles a deep blackish purple and the protoplasmic envelope a faint violet, much resembling the behaviour of the nuclei and protoplasm of tissue cells in this respect. So far as this reaction can be trusted, the sharp differentiation which exists between the matrix or thread and the chromatic particles might be considered a division into nuclear and protoplasmic constituents, especially as in unstained specimens the chromatic particles can be seen and possess a different refraction from the matrix. Moreover, the occasional solid forms take a tolerably uniform hæmatoxylin stain,

indicating—as, indeed, the aniline colours prove—a uniform diffusion of the chromatic material.

From the observed facts of the behaviour of the stained particles, they would appear to differ from the "sporogenous bodies" of Ernst * and Neisser,† although, perhaps, agreeing better with the "metachromatic bodies" of Babes,‡ such as have been described in a number of micro-organisms and occur regularly in the xerosis group of bacteria. It may be questioned whether in our bacillus these bodies are not different from those which they studied, as there is certainly great difference in respect to the resistance which they offer to staining agents. Now, since Bunge # has shown that the "sporogenous bodies" of Ernst have no relation to spore formation, it would seem hardly necessary to mention them, and we should not do so except to repeat in this place that, notwithstanding the possession of many chromatic particles, our experiments make it very probable that the bacillus pyogenes filiformis does not produce spores at all; certainly nothing resembling these structures was ever encountered in cover slips from animals or those prepared from cultures.

Evidences of degeneration, such as vacuolization of the bacilli, were occasionally observed in specimens obtained directly from the animal. In slightly growing artificial cultures, and especially in such as had attained their development upon organs and remained a few days at the room temperature, they were very common. The degeneration of the characteristically dotted forms was somewhat different, consisting of a gradual fading of the whole organism until it was seen with great difficulty even in deeply stained specimens. The chromatic particles became altered in outline, irregularly notched or worm-eaten in appearance, and fragmented, often only mere specks remaining to indicate their former presence. It was noticed, and it was certainly a curious phenomenon, that from the degenerated forms a new growth had taken place in the cultures—

* *Zeitschrift f. Hygiene*, Band v, p. 428.
† *Ibid.*, Band iv, p. 165.
‡ *Zeitschrift f. Hygiene u. Infectionskrankheiten*, Band xx, p. 412.
\# *Fortschritte d. Medicin*, Band xiii, 1895, iv, 20 u. 21.

that is, a characteristic solid outgrowth from such a feebly staining rod was occasionally seen. This indication of the change of morphology in the first generation was unmistakable, and also served to prove that the bacilli in the cultures, notwithstanding the difference in staining properties, were the offspring of the transplanted germs.

Regarding the behaviour of the organism in the tissues a few facts should be emphasized. The bacillus is capable of producing in rabbits all forms of infections, from serous inflammations and abscess formations to extensive necrotizing inflammation and septicæmia. The lesions which it produces varies with the mode of inoculation and the place of localization. In the pleura, pericardium and peritonæum it causes serous and fibrino-serous inflammation; in the cerebral membranes, purulent infiltrations; in the viscera, abscesses and cell necrosis; in the subcutaneous tissues and muscles, extensive necrosis and inflammation. The fibrin formation is not great, notwithstanding the remarkably thick and shaggy character of the pleural and pericardial exudates. Nothing could be more suggestive of a fibrinous inflammation than are these appearances, and yet the histological examination shows that there is a small amount only of actual fibrin (staining in Weigert's stain) and that the shaggy appearance is brought about by the great numbers of felted bacilli which form a false membrane upon the serous membranes in the meshes of which the pus cells are contained. Various kinds of necrosis are produced. Simple hyaline cell death, as in the liver; more extensive hyaline necrosis, as in the voluntary muscles; great fragmentation of cell nuclei, as in the intermuscular septa, subcutaneous tissues, and in localized areas in the viscera; and large, spreading, necrotic foci, much resembling the caseation of tubercle, as in the lungs and brain.

The bacilli show in the lung a marked tendency to invade the bronchi. From the descriptions of the pathological process in this situation it will be recalled that these were greatly affected. The bacilli do not grow nearly so freely in the lung substance as in the bronchi; they give rise to dense masses, often sheaflike in form, which project into their lumina and are covered with an exudate

which may fill the tubes, and in which they again spread. From this fact it may be inferred that the organisms are strongly aerobic in character; indeed, if there were any differences observed in rapidity and extent of growth in the artificial cultures they were in favour of the aerobic tubes.

The endarterial changes which are so common in the lungs and are less frequently met with in the skin, heart, muscle, and brain, are of interest as indicating the short space of time required for their development, the manner in which the new growth appears, and the relation borne by the altered vessels to the pathological process in the viscera. Where there is a direct extension of the destructive action of the organisms to the vessel wall, the damage done the latter is usually great, and the result is not a proliferation of cells in the intima, but actual necrosis of the vessel and thrombosis; on the other hand, when the vessels lie within the affected area or near to a focus of bacillary invasion and are themselves not invaded by the organisms, or exposed to the action of so intense a poison as to cause death of a part of the wall *en masse*, then the proliferative changes are to be observed. In view of the other evidences that have been adduced to show that a part of the action of this organism is attributable to a toxic substance which it forms, it is not difficult to see in the endarterial changes mentioned the operation of the same cause. We think that we have brought evidence in the location of the karyokinetic figures of the participation in this process of proliferation of the most superficially placed intimal cells, perhaps even of the endothelium itself. We were distinctly less successful than Hektoen * in demonstrating a constant layer of flattened endothelial cells upon the surface of the new growth; and, indeed, we even doubt the conclusiveness of this observation as demonstrating the non-participation of the endothelium in the multiplication of the cells. It is conceivable that the shape of the superficial cells might be accounted for in no small degree by the blood pressure to which they were subjected. However, it is not our intention to give a critical review on this sub-

* *The Journal of Experimental Medicine*, vol. i, No. 1, p. 112.

ject, as it has just been done in an excellent manner by Hektoen, to whose paper we would refer.

The observation of the occurrence of great numbers of large mononuclear elements within the lumina of the blood vessels affected by the endarterial changes brings up the question of the function of the vascular endothelium as well as the question of the extent of its participation in the inflammatory phenomena. That the vascular endothelium possesses the power of engulfing particles—micro-organisms and others—the studies of Ponfick,* Neisser,† Touton,‡ Metschnikoff,# Wyssokowitch,‖ and Monti△ have shown. And it has also been rendered probable, through the observations of Neisser and Touton, that when filled with bacilli the endothelial cells may become detached from the vessel wall and be found free in the blood current. Our observation of mitosis in occasional large mononuclear cells within the lumen of the affected vessels lying among other mononuclear and polymorpho-nuclear elements with resting nuclei, makes it not improbable that under certain pathological conditions these cells may be the source of some of the inflammatory elements, perhaps, indeed, of the so-called macrophages.

In passing, it may be well to refer again to another unusual phenomenon which was observed in the lung—namely, the invasion of the cartilages of the bronchi by the bacilli, while the inflammatory cells were rarely seen in this structure. It is also of interest to note that in the cases of pericarditis, although there were no muscular changes in the depth of the heart, yet circumscribed patches of interstitial myocarditis were not infrequently met with in this situation.

It seems probable that in the growth of the bacilli in the tissues of the rabbit a certain order was preserved. The tendency to grow in parallel lines, to form sheaflike masses with a wide base and an acute apex, and frequently to surround in a radial manner a central

* Virchow's *Archiv.*, 1869, Band xlviii, p. 1.
† *Ibid*, 1881, Band lxxxiv, p. 514.
‡ *Ibid*, 1886, Band civ, p. 381.
# Pathologic comparée de l'Inflammation, p. 167.
‖ *Zeitschrift f. Hygiene*, 1886, Band 1, p. 3.
△ *Bollettino della Società Medico-Chirurgica di Pavia*, 1895.

group of cells, was too striking a peculiarity to be passed by. The sheaflike development observed upon the pleura and in the lung, but especially in the uterine glands, and the radiate arrangement in the brain abscesses, occurred with too great frequency to be neglected. Whether this feature will be found to be so constant as to serve for the distinction of this organism in the same manner as the ray fungus is distinguished can only be conjectured.*

This organism must be classed with the bacteria which produce gas within the tissues of the living animal. Unfortunately, attention was not directed to this point early enough in the course of the experiments to observe whether or not it produced gas in the serous cavities. Gas bubbles occurred, however, in the inflamed subcutaneous and mediastinal tissues and in the brain. The relation of the bacilli to the gas cavities was variable. In the brain the margin of the cavity contained a large number of the bacilli; in the subcutaneous tissue the collections of gas might be independent of special accumulations of the organism. The most interesting feature was the production of gas in the closed cavity of the uterus, both in the original and in the experimental cases. Here the walls of certain segments were greatly distended, attenuated, and translucent, and the anatomical structures greatly altered by the pressure to which they had been subjected. Bacilli occurred freely within these segments of the uterus.

The effect of local conditions in determining the localization and development of the bacilli is best shown by the consideration of the behaviour of the pregnant as compared with the non-pregnant uterus. If, in the differences observed, modified circulation can be excluded from playing a definite part, we have then to consider that the new anatomical and physiological processes introduced by the pregnancy are responsible for them. It is suggestive, to say the least, to view this fact in the light of the favourable medium which the rabbit's fœtus, as compared to the uterus, afforded for the growth of the micro-organisms.

* It is interesting to note that the cladothrix (see below) which Eppinger obtained from a case of pseudo-tuberculosis and brain abscess in a human being showed a similar palisade-like form of growth in the tissues.

Although leucocytes were abundantly attracted to the inflamed parts in the rabbit, the inclusion of bacilli by cells was an unusual phenomenon. In guinea-pigs, which proved to be distinctly less susceptible to the organism, phagocytosis occurred to a not inconsiderable extent. We have already drawn attention to what we have denominated pseudo-phagocytosis, by which is meant the invasion of dead cells by bacteria, producing appearances which might be mistaken for phagocytosis. This occurred in sterile organs removed from the body which had been used for growing the bacilli.

Other laboratory animals than those noted did not prove susceptible to inoculations with even large quantities of infectious material containing this organism. Thus, in the mouse both subcutaneous and intrapleural inoculations, in the pigeon subcutaneous, intramuscular, and intraperitoneal injections, and in the dog intravenous inoculations, produced no noticeable effects.

The pleomorphic micro-organism described in the foregoing pages has not, so far as we have been able to examine the literature, been described before, nor has it been met with again by us in the year which has elapsed since its first detection. It remains, therefore, to be seen whether its occurrence is so exceptional as these facts would indicate. However, it is not improbable that it has been seen in cover-slip preparations by others who, owing to its refractoriness in this respect, may have failed to cultivate it. The name which we propose for it is intended to cover, in the first place, its most striking morphological feature, and, in the next, its chief pathological effects.

Review of the Literature.

If we except the micro-organism which is believed to cause the different forms of actinomycosis, the organism of madura-foot disease, and the various varieties of proteus, the number of pleomorphic and more highly developed bacteria which have been found associated with pathological processes in human beings and animals is reduced almost to nil, notwithstanding that complex bacterial forms, such as cladothrix, crenothrix, streptothrix, etc., are widely distributed or-

ganisms in Nature. These have, so far as is known at the present time, played a very inconsiderable part in the causation of pathological conditions, either in man or in the higher animals. It is this fact which lends to the present organism a greater and perhaps more general interest than it otherwise would command. If we exclude diseases produced by the actinomyces organism, the proteus bacilli and the micro-organism of madura-foot disease, and, furthermore, disregard, for the moment, the fact that the bacillus tuberculosis and the bacillus diphtheriæ * have been shown sometimes to possess a more complex structure than had hitherto been ascribed to them, we can quickly review the literature of the remaining pathological processes associated with the pleomorphic and more highly organized bacillary forms.

Rosenbach,† in 1887, drew attention to the relation which existed between erysipeloid, or, as it is also known, erysipelas chronicum and erythema migrans and a cladothrix form of bacterium. The infectious agent, according to him, is found in animal substances which are undergoing decomposition, thus exposing to infection with it such persons as are employed in handling partly decomposed articles. The disease exists among cooks, dealers in game, butchers, tanners, fish handlers, oyster shuckers, and finally dealers who trade in cheese, herring, etc. As the infected material comes in contact almost exclusively with the hands, the affection is found especially upon the fingers, and, as might be expected, other parts of the skin may become infected secondarily from this source. Extending from the point of inoculation there is a deep-red, often livid swelling, with a sharp periphery much resembling the erysipelatous rash. The general bodily health and the temperature are not affected. The disease does not run a definite course and generally comes to a spontaneous end in one, two, or three weeks. Rosenbach obtained from cultures taken from the affected skin a micro-organism which he identified as a form of cladothrix. He found that it grew best upon gelatin at a low temperature, and was able to produce by the inoculation of

* Fischel, Coppen-Jones, Bruns, C. Fraenkel.

† *Archiv f. klinische Chirurgie*, 1887, p. 346.

his own arm with such a culture a condition similar to the natural erysipeloid.

Naunyn* found a peculiar vegetable micro-organism in the hæmorrhagic infiltrated cerebral membranes and upon the endocardial excrescences in a case of chorea. The organism consisted of threads which possessed a brown colour, owing to impregnation with salts of iron. It was regarded by Neumann and Baumgarten as belonging to the threadlike fungi, and by Zopf as occupying an intermediate position between the cladothrix and leptothrix forms of micro-organisms. It must be admitted that this case is not perfectly clear in its interpretation, particularly as Baumgarten and Neumann have drawn attention to the fact that at the time of the isolation of this organism the water-supply which had been used for washing off the organs from the case contained large numbers of leptothrix and cladothrix forms which resembled in appearance those which Naunyn found in the infiltrated cerebral membranes and upon the heart valves.

A number of publications upon the ætiology of pharyngomycosis leptothrica (Chiari) have appeared, from which it is rendered probable that the condition first described by B. Fraenkel under the name of mycosis tonsillaris (pharyngomycosis) benigna is due to the invasion of the affected structures of the throat by a leptothrix bacterial form, possibly the leptothrix buccalis.

Rabe† described a cladothrix which he obtained from purulent phlegmons in the dog. He observed in two dogs a diffuse inflammation of the skin and subcutaneous tissues of one of the fore paws which underwent partial necrosis in the first, and gave rise to suppuration of the glandulæ cervicales superficiales in the other. The latter abscesses were surrounded by granulation tissue of rather peculiar appearance. A third animal, which about three months previously had had an abscess of the parotid gland, died four days after a subsequent illness of an acute peritonitis. In the last animal, as well as

* *Mittheil. a. d. med. Klinik zu Königsberg in Pr.*, 1888, p. 296. (Ref., Baumgarten's *Jahresbericht*, Band iv.)

† *Berl. thieraerztl. Wochenschrift*, 1888, p. 65. (Ref., Baumgarten's *Jahresbericht*, Band iv.)

in the two previous ones, Rabe found in the pus many grapelike, lobulated, pale granules, which upon magnification resembled the actinomyces organism. Cultivation experiments failed, but the inoculation of a goat and of rabbits gave, according to Rabe, positive results. It was possible, by the use of infected material, to produce local abscesses which, however, usually healed, but in one instance caused the death of the experimental animal in six days. In the last case only was the organism recovered from the site of inoculation.

Schmorl* has described a pathogenic, threadlike bacterium (streptothrix (?) cuniculi) which he obtained from the rabbit. He found it in an infectious disease of these animals which began in a rapidly spreading necrotic inflammation of the lips and subcutaneous tissues, and which give rise later to inflammations of the serous membranes (pleura, pericardium, and peritonæum) and also of the lungs. He regarded the organism as belonging either to the group of leptothrix or cladothrix. He succeeded in obtaining it in pure culture upon blood serum where it grew only under anaerobic conditions. Inoculated upon rabbits, it gave rise to the same pathological conditions as in the cases of spontaneous infection. In addition to rabbits, the only other laboratory animals which were susceptible were mice; guinea-pigs, dogs, cats, pigeons and hens having shown themselves refractory to the inoculations. Schmorl concludes that in the bodies of human beings and of the guinea-pig this micro-organism will grow only if it is associated with pus-producing bacteria which prepare for it favourable conditions of growth; alone, it is pathogenic neither for man nor for guinea-pigs.

Eppinger,† in 1890, made the interesting observation that cladothrix forms other than actinomyces might be pathogenic for human beings. He reported the case of a man fifty-two years old in whom he found at autopsy a chronic brain abscess which had perforated into the right lateral ventricle and had given rise, in addition, to a purulent cerebro-spinal meningitis. In the pus and in the walls of the abscess, as well as in the miliary purulent foci in the adjacent

* *Zeitschrift f. Thiermedicin*, 1891, p. 375. (Ref., Baumgarten's *Jahresbericht*, Band vii.)
† Zeigler's *Beiträge zur pathologischen Anatomie*, Band ix, p. 287.

brain substance, many threadlike and apparently branching organisms arranged in groups and in bundles occurred. Lesions resembling tubercles which were in part calcified and in part fibroid existed in the lungs. The bronchial glands were enlarged and calcified, and the supraclavicular glands of the right side were in the same condition. Although this peculiar organism could not be found in the tubercles in the lung, fragments of the threads were discovered among the detritus of the bronchial as well as of the supraclavicular glands. Eppinger cultivated the cladothrix from the pus of the brain abscess upon various culture media. It grew best upon sugar agar and upon blood serum; upon gelatin it grew faintly, but upon potato and in bouillon the growth was regarded as quite characteristic. All the cultures gave the same result upon microscopical examination, and showed the presence of apparently branching threads of various lengths, the apical ones of which resembled cocci. Long threads without branches and smaller bacterialike, cuboidal cells occurred among the branching forms. The first possessed a gliding, and the cubical cells a rapidly whirling or rotary movement. Eppinger observed, in hanging drops containing the small cubical cells, a development of the apparently branching threadlike forms. Experiments upon guinea-pigs and rabbits led to the production of widespread pseudo-tuberculous nodules in which the same organism was again found, and from which it could be obtained in pure culture. Owing to the peculiar starlike manner of its growth, which in tissues much resembles the arrangement observed in the brain abscesses of our experimental animals, the name of cladothrix asteroides has been proposed for the organism by its discoverer.

This brief review comprises the cases in the literature, so far as they have come to our notice, in which pathogenic effects have been observed in man and animals, with the exceptions mentioned, through the action of the pleomorphic and highly organized bacteria. The list is still small; and of this number not all have fulfilled the requirements for admission to the ranks of the recognised pathogenic micro-organisms. It is considered that the proof is con-

clusive of the pathogenic character of the organism described in these pages.

In conclusion, I wish to express my indebtedness to Dr. Welch for many valuable suggestions during the progress of this study, and to thank Dr. Hoen for the photomicrographs which he kindly made.

Explanation of Plates.

Plate IX.

Fig. 1.—Photomicrograph of bacilli derived from the exudate within the uterus of the spontaneous case. Gentian-violet and acetic-acid method of staining. Leitz, one twelfth in. homogeneous immersion objective.

Fig. 2.—Photomicrograph made under the same conditions as the preceding of a cover-slip preparation obtained from a culture upon the fœtus of the rabbit.

Plate X.

Fig. 3.—Experimental abscess of the brain of the rabbit, showing the central mass of pus cells and the peripheral zone of bacilli. Leitz objective No 3, ocular No. 3. Gentian-violet staining.

Fig. 4.—Edge of the previous specimen under the magnification given by Leitz, one twelfth in. homogeneous immersion objective, ocular No. 3.

Fig. 5 shows the sheaves of bacilli in the glands of the uterine mucosa and single bacilli in the stroma of the mucous membrane and in the muscle. Leitz objective No. 6, ocular No 3.

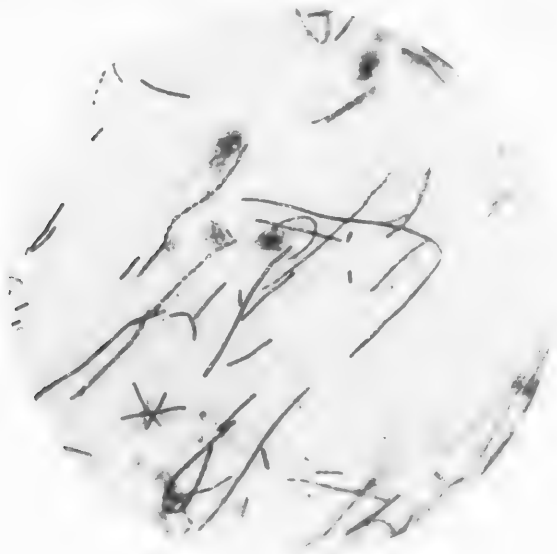

FIG. 1

FIG. 2

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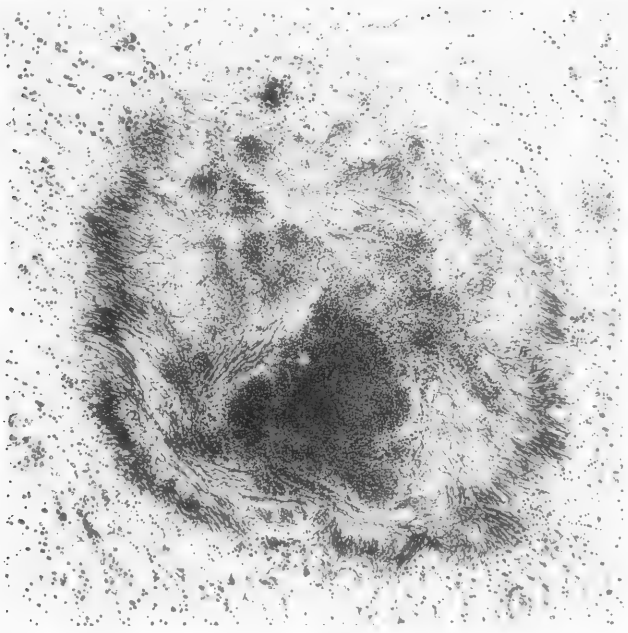

FIG. 3.

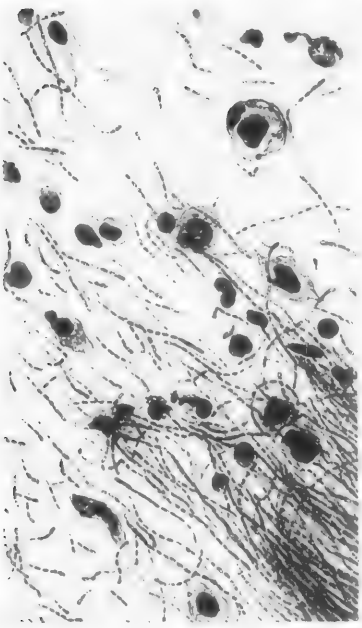

FIG. 4.

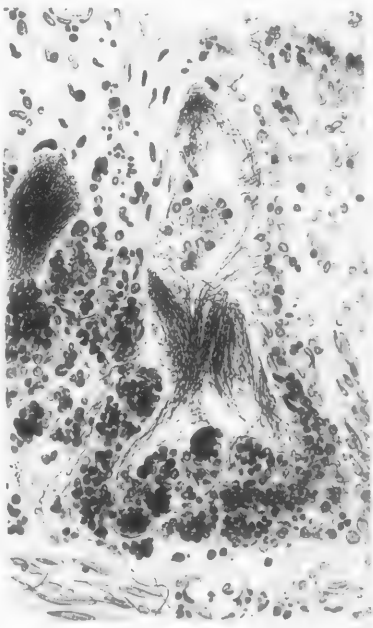

FIG. 5.

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OBSERVATIONS ON THE INNERVATION OF THE SUBLINGUAL AND SUBMAXILLARY GLANDS.

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PLATE XI.

In a research published by Retzius[1] in 1880, which gives the detailed results of an examination of the various ganglia found in connection with many of the cerebral nerves, the jugular and cervical ganglia of the vagus, the jugular and petrosal of the glosso-pharyngeal, the geniculate of the seventh and the Gasserian of the fifth, are described as presenting a structure identical with that of the spinal ganglia. The acoustic ganglion, while it presents some structural peculiarities, was also classed with this group. All other head ganglia—namely, the otic, spheno-palatine, ciliary, and submaxillary—are described as containing cells resembling those of the sympathetic system. The above observations were made on teased preparations, which no doubt accounts for the fact that they did not receive the general recognition due them.

Thanks to the Golgi method, doubts concerning the shape of a nerve cell need no longer be entertained. This method has shown that cells of the spinal ganglia possess a round or oval body, from which springs a single process which divides into two branches a short distance from the cell body, while the sympathetic cells are multipolar, having one axis-cylinder process and numerous protoplasmic branches. The two types are not to be mistaken.

Accordingly we find that nearly all the "head ganglia" have been subjected to renewed investigation. Van Gehuchten[2] has shown that, when stained with the Golgi method, the Gasserian ganglion and the ganglia on the vagus and glosso-pharyngeal, "are comparable in all points to the spinal ganglia," and Lenhossék[3] that the cells of the geniculate ganglion are of the same type. In the acous-

tic ganglion, when impregnated with chrome silver, bipolar cells were found by Retzius[4], Van Gehuchten[2], Cajal[5], Ayers[6], and Lenhossék[7]. Of the other head ganglia studied with the chrome-silver method, the spheno-palatine (Lenhossék[8]) and the ciliary (Retzius[9]) were found to be sympathetic in nature, containing multipolar cells.

This leaves, then, the submaxillary and the otic as the only ganglia found in connection with the cranial nerves which have not been stained by the Golgi method. It seemed to me desirable, in view of the physiological interest attached to the so-called submaxillary ganglion and the nerves in connection with it, to submit it to more careful study; to determine, if possible, the character of its cells and their relation to incoming and outgoing nerve fibres.

Methods.—Two methods have been used—the double Golgi-Cajal method and the Ehrlich-Bethe methylene-blue method. When using the former, the tissues were placed for three or four days in the osmium-bichromate solution, then in three-quarter-per-cent silver nitrate for three days, again in the osmium-bichromate mixture for two days, and finally for three or four days in a three-quarter-per-cent silver-nitrate solution. They were then hastily dehydrated, embedded in collodion, and cut. The sections were cleared in oil of bergamot and xylol and mounted under cover glasses in Canada balsam. I wish to lay stress on the last-mentioned step, as there seems yet a general belief that when preparations are so mounted they are destined to fade in a short time. This is certainly not the case if after placing the sections on the slide the xylol is thoroughly removed with filter paper, and after covering them with a relatively large amount of balsam, the slide is carefully heated over a flame for one or two minutes, or until so much of the solvent has been driven off that the balsam "sets" as soon as it cools. Before cooling, a warm cover glass is placed over the sections. My experience shows that Golgi sections mounted in this way will keep four years without in the least fading.

The Ehrlich-Bethe method was used in the following way: The fresh tissues were stained for about an hour in a one-per-cent solution of methylene blue in normal salt solution, fixed, as Bethe[10]

recommends, in a solution of ammonium molybdate, embedded in paraffin, cut, and mounted.

The following observations were made on specimens obtained from young dogs, and from puppies varying in age from one to ten days. In each animal I exposed the sublingual and submaxillary glands, their respective ducts, the chorda-lingual nerve and triangle. The chorda-lingual triangle (this term is used after Langley [11] and refers to the triangle of tissue between the lingual nerve, chorda tympani, and submaxillary duct), with the underlying sublingual gland and the oral mucous membrane mesial to it, was excised as one piece; the remaining portion of the submaxillary duct, and a thin segment extending through the entire thickness of the submaxillary gland, embracing its hilum, the primary divisions of Wharton's duct, and such lobular ducts as would fall within the segment, as another piece. The tissues so removed were then treated as above mentioned.

Histological Observations.—It is necessary, in the first place, to state that it is not quite correct to speak of a submaxillary ganglion in the sense now generally used, for, as Langley [11] has shown, there are found in the chorda-lingual triangle quite a large number of groups of ganglion cells; the so-called submaxillary ganglion is only one, perhaps the largest, of this number. This statement I can fully confirm. He further points out that these groups of ganglion cells have no connection with that portion of the chorda tympani which goes to the submaxillary gland. This statement he supports both with histological and physiological data. He suggests that "what has hitherto been called the submaxillary ganglion would more correctly be called the sublingual ganglion," and in speaking of the group of ganglia found in the chorda-lingual triangle I shall adopt this nomenclature.

Langley's [11] observations as to the ganglia found in connection with the submaxillary gland are as follows: "Very soon after the submaxillary duct has run free from the sublingual, nerve cells are found upon the medullated bundles which accompany it. The nerve cells are in greater number and form a ganglion much larger

than the so-called submaxillary ganglion." And again: "It is well known that nerve cells occur here and there in the course of the nerve fibres round the lobular duct." I gather, from an examination of preparations made from a large number of glands, that several fairly large groups of ganglion cells are found in connection with the chorda tympani (as it follows Wharton's duct) at a point where it is about to enter and just after it enters the gland, smaller groups being in connection with such of its branches as enter the gland and accompany the larger gland ducts. These observatons verify in the main those quoted from Langley. When necessary to make mention of this group of ganglia, it will be referred to as the submaxillary ganglion.

In all preparations stained after the Golgi method in which the impregnation was successful the nerve cells of the sublingual and the submaxillary ganglia are multipolar in type. As may be seen from Plate XI, Fig. 1, which is a reproduction of the cells stained in one of the smaller ganglia found in the submaxillary gland, the processes are given off largely from one side of an oval or round body. They are usually quite short, often dividing into two or three secondary branches which interlace with similar processes from other cells. It is not unusual, however, to find here and there a cell (Plate XI, Fig. 1, *a*) from which a process is given off, which can be traced for a longer or shorter distance in the field, and which, when viewed a short distance from the cell, is, as a rule, beset with varicose enlargements and does not branch. There can be no doubt that we are here dealing with the axis-cylinder process, while the others may be looked upon as protoplasmic branches. The cells of the sublingual ganglion present the same appearance.

A comparison of the cells here sketched and described—with the descriptions and diagrams of sympathetic cells, found in the reports of investigators, who have in recent years turned their attention to the study of the sympathetic system with the Golgi method—will show that the cells of the sublingual and the submaxillary ganglia are sympathetic in nature, the reports of Kölliker[12], Cajal[13], Van Gehuchten[14], Retzius[15], Sala[16], and Lenhossék[8] showing that

sympathetic cells are multipolar, with one unbranched axis-cylinder process.

Mention may here be made that Retzius[17] has described and diagrammed small groups of sympathetic ganglion cells found by the side of the larger ducts and blood vessels of the submaxillary gland. He was not, however, able to determine the fate of their processes.

The question may now be asked, What is the distribution of the axis-cylinder processes of the sympathetic cells of the sublingual and submaxillary ganglia? The account gathered from an examination of preparations made from submaxillary glands stained after the Golgi method will here be presented; the conditions here are somewhat simpler, as many of the groups of ganglion cells belonging to the submaxillary ganglion are found in the gland itself, which is not the case for the sublingual gland and ganglion. The small groups of ganglion cells are found in the immediate neighbourhood of the lobar and lobular ducts; I have not found any on the walls of the vessels. From such a ganglion small bundles of thin nerve fibres, many of which are varicose and into which one can now and then trace the axis cylinder of a sympathetic cell of the ganglion, can be followed for a longer or shorter distance along the side of the duct on which the ganglion is situated. In successive sections of a series, small bundles will be found accompanying the ducts, and these or similar bundles can be traced further and further into the lobules of the gland. About the intralobular ducts, especially the smaller ones, there is found a toleraby dense plexus of fine nerve fibres. From the plexus strands of two, three, or more fibres, or single fibres, are given off, and can be followed between the alveoli, where they unite to form a perialveolar or epilamellar (Arnstein) plexus (Plate XI, Fig. 2). It seems superfluous to state that the ducts of a gland like the one under discussion branch in such a way that it is quite out of the question to cut sections any single one of which contains a portion of the submaxillary duct, one or several of its primary branches, and even one of the intralobular ducts, with its system of end branches and alveoli. When, in addition to this, the precariousness of the Golgi method is taken into consideration, one would hardly expect to find

sections in which the sympathetic cells were successfully stained, the axis cylinders of which can be followed along the larger and smaller ducts to their ultimate distribution about the alveoli. In a few instances, however, an axis cylinder from a sympathetic cell, which was observed accompanying a duct for a short distance, could be followed as it left the duct and approached a group of alveoli; and in one instance, in a section of the chorda-lingual triangle, which also contained a portion of the sublingual gland, two sympathetic cells deeply stained were seen, the axis cylinder of one of which could be followed for quite a distance by the side of an intralobular duct, and after a short interruption a fibre of the same size and appearance could be traced into a perialveolar plexus. I am well aware that the account here presented is fragmentary; the instance last mentioned seemed, however, so conclusive that I feel justified in stating that the axis cylinders of the sympathetic cells accompany the ducts and ultimately form a plexus about the alveoli.

Retzius described in 1888 a plexus of fine fibrillæ about the alveoli of the "small salivary glands" found in the tongue of a rabbit near the papilla foliata. These observations were made on preparations stained in methylene blue. The following year Ramón y Cajal[18], in a brief notice on the ending of the nerves in the salivary glands, gave the results as obtained with the Golgi method. He here states that "in the submaxillary gland of the rat and rabbit the nerve fibres are arranged in the form of a plexus with round or polygonal meshes. The bundles of this plexus differ in thickness, often have a wavy course, and are composed of non-medullated axis cylinders varying in size. The axis cylinders divide during their course, and give off fine varicose fibrillæ which seem to end on the membrana propria or on the outer surface of the cells." Retzius[17], in a later publication on the basis of renewed investigation with the chrome-silver method, states that he is able to verify in full his earlier observations, and confirms the results obtained by Cajal. Regarding the ultimate distribution of the fibres of the perialveolar plexus and their relation to the cells of the alveoli there is as yet a divergence of opinion. Retzius[17] states that "he was unable to

determine whether the nerve fibres terminated on the outer surface of the membrana propria or between it and the gland cells; certain it is that they end very near the gland cells, and it is very probable that they come in direct contact with them." Cajal, as above quoted, traces the ultimate fibrillæ to the outer surface of the salivary cells.

Arnstein[19] has recently reported on some work which he and his pupils have been carrying on with the methylene-blue method. He himself has investigated the pancreas, the salivary and Harder's glands, Timofeew the prostate, Ostroumow the skin glands, and Dmitrewsky the mammary gland. In a *résumé* Arnstein expresses himself as follows: "In all tubular and acinous glands one finds an epilamellar network which lies on the membrana propria; from this fine fibres are given off which pass through the membrana and, as pericellular threads, come in contact with the gland cells. These fibres form no network under the membrana; they may divide, and end in varicose filaments, the configuration of which differs in different cells."

In some of my preparations, in which the perialveolar plexus was well stained with chrome silver, I now and then find very fine varicose fibrillæ given off from it. They often present a very irregular course, now and then are seen branching, and seem to end on or between the gland cells. They lie in a plane a little deeper than the perialveolar plexus, are much finer than its fibres, and more often show a beaded appearance. As far as my observation goes, these ultimate fibrillæ, which seem to correspond to the pericellular branches which Arnstein[19] and his pupils find below the membrana propria, end in very small nodules or granules. I have not seen the more complicated ending described by Arnstein.

The facts gathered from the literature reviewed, and the observations made by myself, may be expressed in the following brief summary: The axis-cylinder branches of the sympathetic cells found by the side of the ducts of the submaxillary gland are grouped into small bundles which accompany the branches of the ducts to the lobules and here form a plexus about the intralobular duct, from which branches are in turn given off which form a second plexus on

the membrana propria about the alveoli; from this plexus fibrillæ penetrate the membrana propria and end on the gland cells.

We may now turn to the chorda tympani. Langley [11] has shown that "it consists of four or five small bundles of medullated nerves. . . . Besides these, several other small bundles of fine medullated fibres are given off by the lingual in its course between the point where it gives off the chorda tympani proper and where it crosses the duct of the submaxillary gland. These bundles are given off from both sides of the lingual nerve; after a very short course, nerve cells occur within the sheath of the nerve bundles; the nerve cells are scattered among, though often collected chiefly on one side of, the medullated nerve fibres; on the distal side of the nerve cells non-medullated fibres are found, and the medullated fibres are reduced in number." And again, after describing the location of the ganglion cells in the hilum of the submaxillary gland, as previously quoted, he states that "from the distal end of this (submaxillary ganglion) issue bundles which contain comparatively few medullated fibres." The non-medullated fibres issuing from the distal portion of the groups of ganglion cells of sublingual and submaxillary ganglia are no doubt the axis-cylinder processes of the sympathetic ganglion cells of the several ganglia, concerning which mention has above been made.

In some of the Golgi-stained preparations made from the chorda-lingual triangle and submaxillary gland, in which the ganglion cells are not at all or only imperfectly stained, and in others stained in methylene blue, I find a very dense plexus of fine varicose fibrillæ surrounding the ganglion cells. This plexus is woven into a basket-like structure, and into it one can at times trace a rather coarse axis cylinder. This pericellular network I have found a great number of times, and in various ganglia of the sublingual and submaxillary group.

In methylene-blue preparations the fibrillæ of the plexus are of a violet-blue colour, on which more deeply stained granules are discernible. The inclosed ganglion cell, with its processes, either remains unstained, or only the cell body and nucleus are tinged a very light blue.

In Golgi-stained preparations this plexus is not seen when the cells are stained. In some of the preparations in which the cells remained unimpregnated beautiful examples were found. The shape and arrangement of this basketlike network was essentially the same in methylene-blue and chrome-silver preparations, except that in the latter case the fibrillæ appeared somewhat coarser. One such pericellular basket is reproduced in Plate XI, Fig. 3; it was taken from one of the small ganglia of the submaxillary gland in a preparation stained after the Golgi method. A rather coarse axis cylinder can with some difficulty be followed between some other stained fibres, and, as the figure shows, divides into a number of branches (at *b*), which, after further branching, anastomose to form the network.

Does this pericellular network indicate the ending of the nerve fibres of the chorda tympani? The following reasons suggest themselves for assuming that it does:

1. Langley[11] has shown that medullated fibres approach the ganglia and a great number of non-medullated fibres are given off from their distal end.

2. In cases where it was possible to determine the direction of an axis cylinder ending in a pericellular network its course was toward the centre.

3. Axis cylinders terminating in a pericellular network are usually somewhat coarser than the axis-cylinder processes of sympathetic cells which are often found beside them; their course is more regular, and in methylene-blue preparations I have a few times recognised the presence of a sheath of myelin at a short distance from their termination in a pericellular network.

4. It is a well-known fact in physiology that stimulation of the chorda-tympani causes an increased flow of saliva. Langley and Dickinson have shown that "nicotine in moderate doses paralyzes the nerve cells in sympathetic ganglia without paralyzing the peripheral ending of nerve fibres." Langley[11] finds that "when a certain amount of nicotine is injected into the vein of a cat, or of a dog, stimulation of the chorda-lingual nerve causes no secretion"; this, as he states, has also been observed by Heidenhain. If, how-

ever, the electrodes are pressed into the hilum of the submaxillary gland and strong tetanizing shocks are used, a secretion nearly as rapid, as when the chorda lingual is stimulated before the injection of nicotine, is obtained. He further shows that the flow of saliva is not due to stimulation of the sympathetic nerves about the submaxillary artery, as after the injection of atropine in sufficient quantity to paralyze the chorda, but insufficient to paralyze the sympathetic, stimulation at the hilum of the gland gives little or no flow. From this Langley concludes that "nicotine paralyzes the nerve cells on the course of the chorda-tympani fibres."

In methylene-blue-stained preparations axis cylinders can often be traced through a ganglion without terminating in a pericellular end-basket. They no doubt are the few medullated fibres which, in conjunction with non-medullated fibres, issue from the distal portion of ganglia. We may assume that such medullated fibres end in pericellular networks in more distant ganglia, as among the fibres composing the network about the intralobular ducts no medullated fibres are seen.

It would seem that now and then the axis cylinder of a medullated fibre branches and forms a network about more than one cell. I have seen one example where an axis cylinder divided into two branches, each of which terminated in a pericellular network.

That there exists a network of fine nerve fibres about sympathetic cells is now well known, and has been shown by a number of investigators. In Ehrlich's [20] first publication concerning the reaction of methylene blue on living nerve tissue he speaks of the sympathetic cells (presumably in the frog) as bipolar, with a straight and a spiral process. In his preparations only the spiral process was stained. This he describes as dividing and forming a network which wholly or partially incloses the cell body. From this network fine fibrillæ were given off which terminated on the cell in small enlargements. Two years later Smirnow [21] published his observations on the structure of sympathetic cells of amphibia. The sympathetic chain and the cells found in the pharynx, lung, septum auriculi, and bladder of frogs and toads were investigated. His results were in the main con-

firmatory of those obtained by Ehrlich, with this exception: that he was not able to see the free ending of fibrillæ given off from the pericellular network, as described by the originator of the methylene-blue method. After discussing at some length the nature and course of axis cylinders ending in the network, Smirnow[21] makes the following statement, which I quote in full:

"*Auf Grund der eben mitgetheilten Thatsachen gelange ich zu dem Schluss, dass die Spiralfasern gegen die Peripherie hinziehen und dass sie Nervenfibrillen enthalten, welche letzere je nach dem Fundort der Nervenzellen zu verschiedenen peripheren Gebilden in Beziehung treten.*"

That these conclusions are erroneous is shown, I think, in what has already been said in giving the reason for believing that the pericellular network about the sympathetic cells of the sublingual and submaxillary ganglia are the terminal endings of the chorda tympani. As there stated, the fibres ending in the baskets extend centralward and are not distributed peripheral to the ganglia. Aronson[22] has described a similar pericellular network about the sympathetic cells of the neck, and the abdominal and cardiac ganglia of the rabbit; and Timofeew[23] about the ganglion cells found in the epididymis.

Lenhossék[8] describes and pictures one very typical pericellular basket seen in the spheno-palatine ganglion of a newborn mouse treated with chrome silver; he adds that the nerve fibre could be followed only a short distance and extended centralward. Lenhossék also describes free ending of nerves in sympathetic ganglia. Whether this is the case in the submaxillary and sublingual ganglia I can not say, as I am unable to state whether the fine fibrillæ found between ganglion cells are to be interpreted as a free ending or as imperfectly stained end-baskets.

Sympathetic Fibres accompanying the Gland Artery.—It is a well-known fact that sympathetic nerve fibres accompany the submaxillary artery to the gland. Langley[11] has shown that "up to the distal end of the ganglion in the hilum—that is, the real submaxillary ganglion—they are unconnected with the plexus resulting

from the chorda tympani, and have no nerve cells on it. This gives ground for concluding that sympathetic nerve fibres are not connected with nerve cells which occur in the course of the chorda tympani." Langley [11] has also shown that when sympathetic cells are paralyzed by the injection of nicotine into the veins of cats, rabbits, and dogs, or after the application of this drug to the superior cervical ganglia, stimulation of the sympathetic in the neck causes no secretion, but that a secretion is obtained when the ganglion or the nerve fibres coming from it are stimulated. He concludes that "the secretory fibres of the sympathetic are not connected with nerve cells beyond the superior cervical ganglion."

The following observations were made on preparations stained in methylene blue and in chrome silver:

Fine varicose fibres, woven into a plexus, were found on the walls of vessels in submaxillary and sublingual glands. Ganglion cells in connection with such fibres have not been observed; neither could they be traced into ganglia found on gland ducts (submaxillary gland); nor were they observed ending near gland cells. These statements would confirm the results obtained by Langley. On putting these facts together, I conclude that the sympathetic fibres going to the sublingual and submaxillary gland with the gland arteries end on the vessels.

One other observation may here receive brief mention—namely, the ending of nerve fibres in the submaxillary and sublingual ducts. These ducts are surrounded by a loose plexus composed of small bundles of nerve fibres. From the plexus axis cylinders are given off which, after repeated division, approach the epithelium, ending therein in very fine varicose fibrillæ.

Plate XI, Fig. 4, shows such an ending in the submaxillary duct. It was taken from a preparation stained in methylene blue. As may be seen from the figure, the ultimate fibrillæ end between the cells of the epithelium. The intra-epithelial branches are usually quite straight, end in small enlargements, and often extend to very near the free ends of the cells between which they are found.

Plate XI, Fig. 5, expresses by way of diagram the general con-

clusions reached. It is based on observations here recorded and on facts gathered from Langley's admirable paper on "salivary secretion," from which I have so largely drawn.

The sublingual and submaxillary ganglia are groups of sympathetic ganglion cells (*f*, *g*). The axis cylinders of these cells (*f'*, *g'*) follow the ducts to the glands and end on the cells of the alveoli (*a* and *b*). The chorda tympani (*d*) contains fibres, some of which (*d'*) end in the sublingual ganglion, others (*d''*) in the submaxillary ganglion. In both ganglia the fibres of the chorda tympani terminate in a pericellular network which incloses the sympathetic cells. The fibres of the chorda tympani going to the submaxillary ganglion have no connection with the nerve cells in the chorda-lingual triangle (*e*, *e'*, *e''*).

The sympathetic fibres (*h'*) which accompany the gland arteries are axis cylinders of sympathetic ganglion cells situated in the superior cervical ganglion (*h*). They end on the blood vessels (*k*, *k'*) of the glands.

Addendum.—After this article was in the hands of the publishers my attention was called to Berkley's * studies of the nerve endings in the submaxillary gland of the mouse.

Berkley's preparations were obtained from tissues stained in chrome silver, prefixed in picric acid. In the main, Berkley's results receive confirmation in the observations recorded by me. The entrance of the nerve fibres with the blood vessels and ducts, the plexus around the ducts, the meshwork and single fibres about the alveoli (perialveolar plexus), and the ending on or between the gland cells, have received essentially the same interpretation.

In the hilum of the gland, "and located near a secretory duct and an artery," Berkley finds "nests of large unstained sympathetic cells." In none of my sections did I ever find ganglion cells in conjunction with the blood vessels, only on the gland ducts, nor was I able to trace any nerve fibres from the ganglia to the blood vessels, as

* The Intrinsic Nerves of the Submaxillary Gland of Mus Musculus. *The Johns Hopkins Hospital Reports*, vol. iv.

he describes. In all preparations where nerve cells and axis cylinders were well stained the axis cylinder was seen accompanying the ducts.

Berkley makes no mention of the pericellular baskets (endings of the chorda tympani) to which especial attention has been drawn in this article.

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FIG. 1

FIG. 2

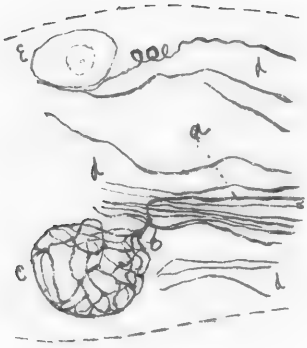

FIG. 3.

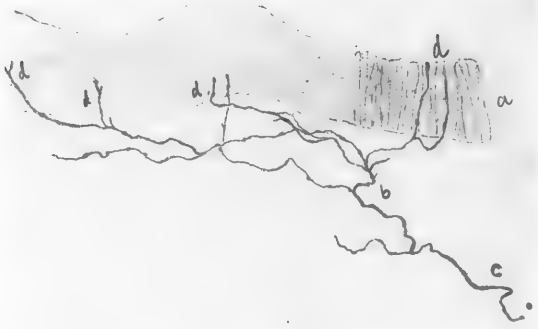

FIG. 4

FIG. 5

16. Sala.—Sulla fina anatomia dei gangli del simpatico. *Monitore zoologico italiano*, Nos. 7, 8, 1892. Reviewed by Golgi in *Ergebnisse der Anat. und Entwicklungsgeschichte*, vol. ii, which is the only account I have seen.

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Description of Figures, Plate XI.

Fig. 1.—Sympathetic ganglion cells in Golgi-stained preparation. One of the small ganglia in submaxillary gland; *a*, cells showing a well-stained axis-cylinder process; *b*, medullated nerve fibres entering ganglion.

Fig. 2.—To show intralobular duct (*a*) surrounded with a plexus of fine axis cylinders; *b*, alveoli with perialveolar plexus. Golgi stain.

Fig. 3.—To show pericellular end-basket; *a*, coarse axis cylinder branching at *b*; *c*, pericellular end-basket; *e*, unstained ganglion cell; *d*, other axis cylinders. Golgi-stained preparation.

Fig. 4.—To show ending of nerves in epithelium of submaxillary duct; *a*, epithelium lining duct; *c*, axis cylinder branching at *b*, and *d*, intra-epithelial fibrillæ. Methylene-blue stain.

Fig. 5.—Diagram of nerve supply of sublingual and submaxillary glands; *a*, lobules of sublingual gland; *b*, lobules of submaxillary gland; *c*, fibres of lingual nerve; *d*, fibres of chorda tympani; *d'*, chorda-tympani fibres ending in chorda-lingual triangle; *d''*, chorda-tympani fibres ending in submaxillary ganglion; *f*, sympathetic-nerve cells of sublingual ganglion; *f'* their axis-cylinder branches; *g*, cells of submaxillary ganglion; *g'*, their axis-cylinder branches; *h*, cells of superior cervical ganglion; *h'*, axis cylinders; *k*, blood vessels of sublingual gland; *k'*, blood vessels of submaxillary gland.

A NEW METHOD FOR THE STUDY OF THE INTRACARDIAC PRESSURE CURVE.

By W. T. PORTER.

(*From the Laboratory of Physiology in the Harvard Medical School.*)

The method about to be described secures a correct record of any part of the intracardiac pressure curve, for it meets the theoret-

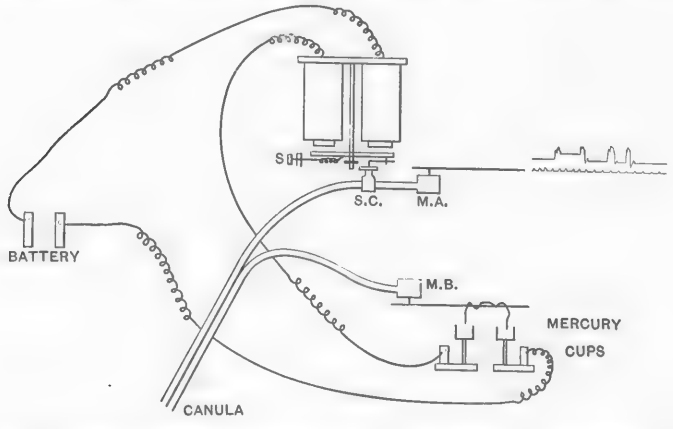

Fig. 1.—Scheme illustrating the arrangement of the apparatus. The meaning of the abbreviations is given in the text.

ical requirements of a perfect method, in that it employs an incompressible fluid and at the same time practically does away with the inertia error.

A short double canula is passed into the left ventricle through the subclavian artery and aorta. One tube of the canula is connected directly with a Hürthle membrane manometer (Fig. 1, M. B.) placed upside down. The recording lever of this manometer bears a wire, the ends of which are thrust into two mercury cups when the rising pressure in the inverted manometer carries the recording lever downward. When the pressure in the manometer

falls, the lever withdraws the wire from the mercury cups. The passing of the wire into the mercury completes an electric circuit, and sends a current through a strong electro-magnet, the armature of which is arranged to open a stopcock (S. C.) placed between the second tube of the canula and a second membrane manometer (M. A.). When manometer B withdraws the wire from the mercury cups, thus breaking the circuit, the magnet releases the armature, and the latter is pulled back into its former position by the adjustable spring S, and so closes the stopcock. During the passage of the current through the magnet, manometer A, being placed by the opening of the stopcock in communication with the cavity of the ventricle, will write a pressure curve. The current, however, will pass through the magnet only when the wire of manometer B is in the mercury, hence the period during which manometer A can write the intraventricular pressure curve is controlled by manometer B.

If now manometer B is so adjusted that the ends of the wire are near the mercury during the diastole of the ventricle, the wire will dip into the mercury and open the stopcock soon after the beginning of the systolic rise of pressure, and manometer A will write the systolic curve almost from its commencement, as in Fig. 3. If, on the other hand, manometer B is so adjusted that the wire during diastole is far above the mercury, the rising intraventricular pressure will not succeed in bringing the wire into the mercury until toward the maximum of systole, and manometer A will write only the summit of the curve, as in the first curve of Fig. 6. Thus the portion of the intraventricular curve that is written by manometer A can be selected at the operator's pleasure merely by varying the position of the lever of manometer B, and thus varying the moment at which the wire shall make the circuit and open manometer A to the ventricle.

The satisfactory working of this method requires an exceedingly sensitive manometer, a stopcock that will open and close with great rapidity, and an entire absence of jar. The stopcock first employed was of glass. The rapidity with which it opened and closed

was measured from a curve drawn by a writing point fastened on the armature of the electro-magnet that moved the stopcock (Fig. 2). It is seen that the time occupied by the entire stroke is less than a fiftieth of a second.

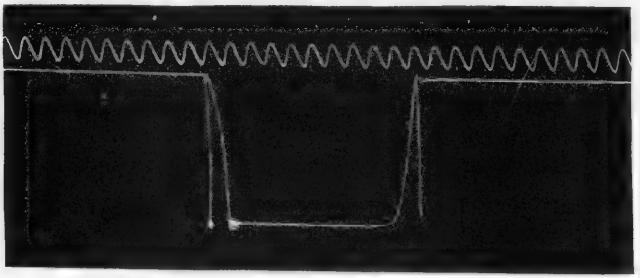

Fig. 2.—The original curves show greater fineness of line than appears in these reproductions. The upper curve was drawn by a rod making fifty double vibrations per second. The lower curve was drawn by a writing point fastened on the armature of the electro-magnet that moved the stopcock. The perpendiculars are drawn at the beginning and the end of the curves.

This glass stopcock was afterward replaced by one of brass,* the speed of which was considerably greater. The exact details of its construction, together with the arrangement and dimensions of the electro-magnet and the mercury cups, will be given in a subsequent paper, as the apparatus is now undergoing modifications by which, it is hoped, its efficiency will be increased.†

For present purposes it is enough to determine that the time lost between the making of the circuit by the wire of manometer B and the commencement of the curve of manometer A is inconsiderable. More than this is shown by the curve in Fig. 3.

An electric signal in the primary circuit wrote the upper curve. The next curve is that of the ventricular pressure, written by manometer A. The lower line marks the atmospheric pressure and the time in fifths of seconds. To the right is a graduation scale in intervals of 20 millimetres, Hg. The manometer, after being raised from

* Figs. 4, 5, and 6 were written with the brass cock and a wholly undamped manometer.

† The electro-magnetic stopcock is a slight modification of one invented by Prof. Bowditch and described by him in *Arbeiten aus der physiol. Anstalt zu Leipzig*, 1871, p. 143.

the atmospheric pressure to 160 millimetres, Hg., was placed again at the pressure of the atmosphere. The return to the abscissa is so perfect that the manometer line is entirely hidden in the line of the abscissa. It has already been said that the elevation in the upper line,

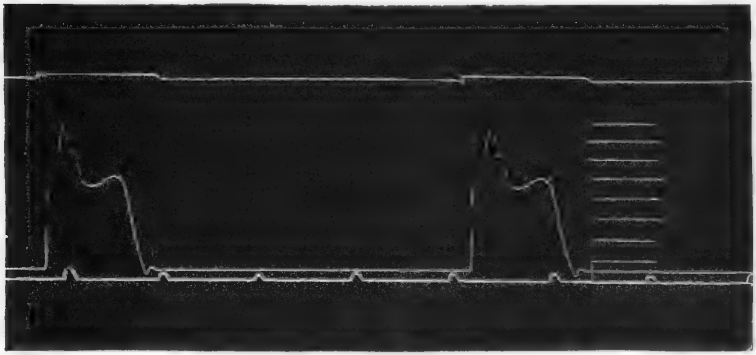

Fig. 3.—The elevation in the upper curve is the period during which the current flowed through the electro-magnet. The lowest line is the atmospheric pressure and the time in fifths of seconds. The graduation scale is in intervals of 20 millimetres, Hg.

written by the electric signal, records the period during which the current passed through the electro-magnet controlling the stopcock. The difference in the length of this elevation and the time during which the manometer wrote its curve is the time lost by the opening of the stopcock and the inertia of position (*Trägheit*) of the manometer, less the interval between the breaking of the circuit and the closure of the stopcock. The measurement of these different components will be given at another time. It is sufficient now to point out that the difference between the time during which the current flows through the stopcock and the time during which the curve is writing is too small to be of any practical importance.

If the manometer B is arranged to open the stopcock near the summit of intraventricular contraction, the first systolic rise after the experiment is begun will show a great inertia error in the curve written by manometer A, for this undamped manometer will be suddenly exposed to a high intracardiac pressure and the lever will be driven

from near the atmospheric pressure to the height of intraventricular pressure in less time—that is, with greater velocity—than is the case in a manometer open at all times to the ventricular pressure. During the interval between the first and second contractions the pressure in the manometer A will not fall very far before the stopcock will close, leaving a high pressure in the manometer. For the manometer B, it will be remembered, was arranged to close the circuit near the summit of intraventricular pressure. It must, therefore, necessarily pull the circuit-making wire out of the mercury, and so break the circuit again, when its lever, in returning toward the abscissa, reaches a height above the abscissa equal to that at which the wire entered the mercury during the systolic rise of pressure. The second contraction of the ventricle will therefore find the pressure already high in manometer A. The rise that manometer A has now to record, when the stopcock opens, is not the difference between the pressure at the beginning and at the summit of systole, but between the summit and a point near the summit—in other words, a fraction of the total difference of pressure in systole. For example, the pressure in the manometer A may have been left at 100 millimetres, Hg.; during the next systolic rise the manometer will need to move, then,

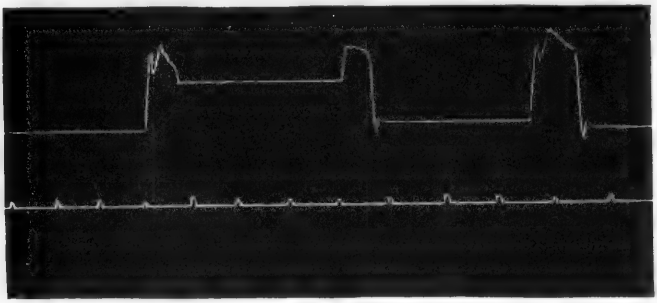

FIG. 4.—The upper line is the curve of intraventricular pressure. The lower line is the atmospheric pressure and the time in fifths of seconds.

only from 100 millimetres to the maximum intraventricular pressure—for example, 150 millimetres, Hg. The inertia error will thereby be much diminished, for the error accompanying the record

of a difference of 50 millimetres is much less than that accompanying the record of 150 millimetres. Such a lessening of the inertia error is seen in the second curve of Fig. 4.

If now manometer B is so arranged that the stopcock opens when the intracardiac pressure has risen to 145 millimetres, Hg., within 5

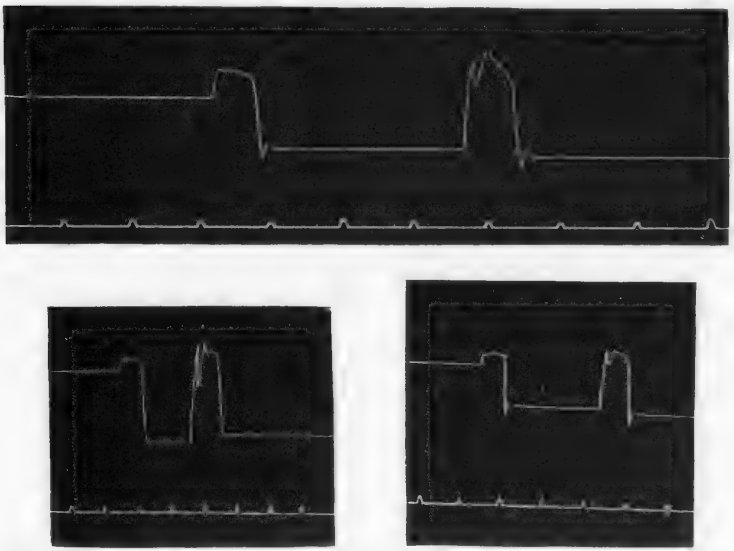

Fig. 5.—The upper line is intraventricular pressure. The lower line is the atmospheric pressure and the time in fifths of seconds. The inertia error diminishes according to the height above the atmospheric pressure at which the manometer begins to write.

millimetres, let us say, of the maximum, then manometer A, after a stroke or two has left its pressure at 145 millimetres, will have but 5 millimetres to record, and the inertia error accompanying the record of 5 millimetres is practically insignificant.

Thus the true summit of the intraventricular curve, unmarred by inertia errors, is written.

But this is not the only advantage of the new method. It is evident that the manometer that will give a useful record of a sudden difference of pressure of 150 millimetres, Hg., can not give a large excursion for a small increment of pressure. Practically it is not

advisable to have the lever rise much above 1 millimetre for each increase of 10 millimetres, Hg., in pressure. Hence fine details, involving changes of a few millimetres, Hg., are apt to be obliterated by friction on the recording surface or other causes. But with the new method such details can be easily studied, because the manometer that is to write only a part of the intraventricular curve—5 millimetres, Hg., at the summit, for example—can afford to give a much larger excursion than the manometer that must record the whole curve.

When the mercury in the cups is at rest, the summits of the intraventricular pressure curves are written with almost schematic uniformity. If, however, the mercury in the cups is shaken by the current of water that is kept flowing over it in order to protect the wire

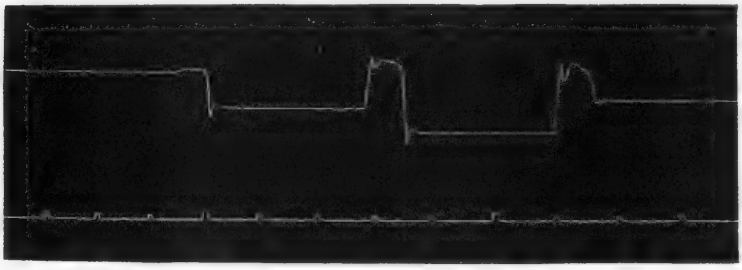

FIG. 6.—Upper curve, intraventricular pressure. Lower curve, atmospheric pressure and time in fifths of seconds. At the left only the very summit of the intraventricular pressure curve is written.

of manometer B from burning away, the make and break no longer occur at precisely the same height above the atmospheric pressure, and the interesting irregularities illustrated in several of the accompanying figures are seen.

It is evident that with this method the long-standing question as to the real form of the summit of the intracardiac pressure curve can be definitely answered. The nearer the summit the recording manometer begins to write, the more "plateau"-like the summit becomes and the less marked are those elevations the meaning of which has provoked so much discussion. They disappear entirely when the

manometer writes the last few millimetres at the highest part of the curve, and the plateau then appears unvexed by inertia oscilla tions.

The summit of the normal intracardiac pressure curve is, as a rule, nearly parallel with the abscissa.

The method here described is applicable to any portion of the intracardiac or other pressure curves. With its aid the portion of the intraventricular curve lying next the atmospheric abscissa line can be studied with especial profit, and information of value, as I hope to show in my next communication, can be secured with regard to the subatmospheric pressures sometimes present in the ventricle.

THE ROTARY PROPERTIES OF SOME VEGETABLE PROTEIDS.

By ARTHUR C. ALEXANDER, Ph. D.

(*From the Sheffield Biological Laboratory of Yale University.*)

I. Introductory.

So far as the writer has been able to ascertain, no reliable determinations have ever been made of the rotary properties of any of the vegetable proteids, although some of them can now be obtained in a pure state and in considerable quantities. The object of this investigation was to determine accurately the rotary power of some of the crystallized vegetable proteids. The globulin found in hemp seed was chosen as the main subject of study, because it could be obtained in a perfectly crystalline form with comparative ease. For purposes of comparison, the rotary powers of the crystallized globulins obtained from flaxseed and the Brazil nut were also determined under similar conditions. The identity of each of these three proteids has been completely established by the researches of Osborne,* Chittenden and Mendel,† and others, and a guarantee of chemical purity is furnished in their crystalline form. Their individual compositions and properties will be described later in connection with the results of the polarimeter observations.

A complete determination of the rotary properties of any substance should include a study of the effect on its rotary power of three factors—(1) the solvent used; (2) the concentration of the solution; (3) its temperature. The proteids investigated by the writer were imperfectly soluble in most solvents at the temperature of the room, and their solutions were more or less coloured. On account of their opacity, only dilute solutions containing about one or two

* *American Chemical Journal*, vol. xiv, pp. 629, 662.

† *Journal of Physiology*, vol. xvii, p. 50.

per cent of proteid matter could be used, so that the angles measured had to be multiplied by a large reduction factor to find the rotation for the standard length and density. The errors of observation were thus multiplied many times. For this reason the results were not sufficiently exact, nor were the variations sufficiently great, to determine the manner in which the rotary power varied either with the temperature or the concentration of the solutions. The maximum range in concentration was from 0·50 per cent of proteid to about 2·50 per cent, and in temperature from 20° C. (below which precipitation was likely to occur) to 70° C., above which coagulation soon took place. The specific rotation, as will be seen later, appeared to decrease with the percentage of proteid in solution. The variation with the temperature was apparently slight, and it seemed useless to try to determine it exactly. In fact, the variation of the specific rotation with the solvent was the only factor that could be determined with any exactness.

Preparation of Solutions.

Salt Solutions.—The salt solutions used as solvents were all prepared in the same manner. A given quantity of the chemically pure salt, dried in a desiccator, was weighed out, dissolved in distilled water, and made up with distilled water to the required volume. The solution was then filtered. The residue of salt, when dried at 110° C., was next carefully determined, several portions of the solution of 20 or 30 cubic centimetres each being dried to constant weight at 110° C., and the results, which never differed by more than 2 or 3 milligrammes, averaged. None of the vegetable globulins experimented upon being completely soluble in these solutions, as concentrated a solution of globulin as possible was made and then filtered clear of the undissolved proteid. The percentage of proteid matter in solution was afterward determined by drying down 20 cubic centimetres or so to constant weight at 110° C., and subtracting from the residue the weight of the included salt. The percentage of ash in the dried proteid having been previously determined by ignition, the weight of ash- and water-free proteid in 100 cubic centi-

metres could easily be calculated. The concentration of the proteid solution was varied by diluting it in a graduated flask with the original solvent.

Acid and Alkaline Solutions.—The globulins studied, except when very dry, can be completely dissolved in dilute acid or alkaline solutions with comparative ease. This simplified very much the preparation of such solutions. The percentage of moisture in the globulin, which was kept in a bottle under fairly constant conditions of temperature and humidity, was carefully determined by drying it at 110° C. Thus, the percentage of moisture and of ash being known, the amount of water- and ash-free matter in a given weight could be readily found. In making the solutions a few grammes of the globulin were weighed out and completely dissolved in the acid or alkali, and the solutions were then made up to the required strength in a graduated flask.

A few alkaline solutions were also prepared by the addition of a definite volume of eight-per-cent potassium hydroxide to a sodium-chloride solution of the globulin. If the alkali is not too weak it can be added to a salt solution in this way without precipitating the globulin. Acids, on the contrary, produce precipitation immediately when added to such a globulin solution.

The 0·2-per-cent sodium-carbonate solution used as a solvent was made in a similar manner to the neutral salt solutions, the sodium carbonate being weighed out and dissolved in distilled water. The other alkalis and acids used as solvents were made up approximately to the requisite strength with distilled water, and their exact strength was afterward determined by titration.

Apparatus and Methods of Observation.

The polarimeter observations were made in a large dark room partitioned off in the centre of the building, and a "half-shadow" polarimeter of the Laurent type,* constructed by J. Dubose, of Paris, was employed to measure the rotary power of the proteid so-

* *Dingl. Polyt. Journ.*, vol. ccxxiii, p. 608.

lutions. This was a large instrument designed for use as a saccharimeter and capable of holding tubes 400 and 500 millimetres long, with a 20-centimetre circle divided to half-degrees, and a vernier with magnifying glass reading to 0·05°. Sodium light was alone used, as no other monochromatic light could be found of sufficient intensity. The exact position of the analyzer when the two halves of the field became of the same shade could not be determined by a single setting closer than 0·05° to 0·10°. However, by taking a large number of observations and averaging them, the error of the mean could be reduced to less than 0·01°. In general, from twenty to forty settings were made and readings taken, the position of uniform shade being approached alternately from the right and the left hand. The temperature of the solution in the tube was found by removing the cover and inserting a thermometer immediately after the rotation had been measured. Instead of depending on the result of one such measurement of the rotation, the tube was always emptied and refilled with a fresh portion of the solution and a new series of readings were taken. This was repeated a number of times, and the weighted mean of the results obtained was adopted as the best value.

The specific rotation was calculated by the formula

$$(a)_D = \frac{100}{l\,c}\,a,$$

where a is the angle of rotation measured, l the length of the tube in decimetres, and c the weight of ash- and water-free substance in 100 cubic centimetres. The factor $\frac{100}{l\,c}$ in this investigation varied, according to the percentage of proteid in the solution, from 50 to as large a value as 200. Thus an error of 0·01° in measuring the angle of rotation, a, meant an error of from 0·5° to 2·0° in the value of the specific rotation, $(a)_D$.

Sources of Error.

A careful investigation of all possible sources of error, both in determining the strength of the solutions and in measuring their ro-

tary power, led to the conclusion that by far the most important source of error lay in the observer himself. The values found at different times for a single setting of the polarimeter, each the mean of thirty or forty readings and having a probable error of less than 0·01°, would frequently differ among themselves by 0·05° or more, especially when the intensity of the sodium flame varied or the observer's eyes became fatigued. The error thus introduced into the value of the specific rotation was from two to five per cent, according to the size of the angle measured, while the errors introduced by the methods of determining the percentage of proteid matter in solution, which ranked next in magnitude, never exceeded 0·3 or 0·4 per cent.

To find the probable error of the measured angle of rotation, the probable error of the initial setting with an empty tube was calculated from a large number of readings covering an interval of some days, and the probable error of the mean rotation reading was calculated in each case from the results of a number of independent series of readings. The square root of the sum of the squares of these gives the probable error in the value of the total rotation, and the probable error of the specific rotation is easily calculated from this. In calculating the probable error of a number of measurements of the same angle the abbreviated formula *

$$r_0 = \frac{0{\cdot}8453\ \Sigma\ v}{n\sqrt{n-1}}$$

was used, where r_0 is the probable error of the mean, v the deviation of each observation from the mean, Σ the usual sign of summation, and n the number of observations.

II. Hemp-seed Globulin.

Preparation and Composition.

The globulin from hemp seed can be easily obtained in a crystalline form in comparatively large quantities. The following method of preparation was employed by the writer:

* See Merriam's Text of Least Squares, 2d ed., p. 93.

The raw, ground hemp seed was treated with a five-per-cent sodium-chloride solution at 60° C. for an hour which was then strained and filtered while hot. The filtrate obtained was rich in globulin, which was precipitated by cooling in the form of perfect but minute crystals. After decanting the supernatant liquid, the precipitate was thrown on a filter and washed successively with distilled water, dilute alcohol, absolute alcohol, and ether, thus removing all salts, fats, and other impurities soluble in water, alcohol, or ether. The ether was evaporated by exposure to the air, and the globulin was finally dried in a desiccator over sulphuric acid. In extracting the globulin in this way, about 50 grammes were obtained on an average from a kilogramme of the ground hemp seed. The crystals examined under a microscope and by polarized light were found to be perfect isometric octahedra with perhaps a few hexagonal plates.

The reactions of this proteid are given in full by Osborne, Chittenden and Mendel, and others.* Its composition has also been carefully determined. Chittenden and Mendel give the following analysis :

Carbon	51·63	Sulphur	0·90
Hydrogen	6·90	Oxygen	21·79
Nitrogen	18·78		100·00

0·8189 gramme of the crystallized globulin prepared by the writer, dried to constant weight at 110° C., yielded, after ignition, a residue of 0·0016 gramme of ash. This gives: Percentage ash = 0·20 per cent.

Sodium-chloride Solutions.

The mode of preparing these solutions has already been described. Hemp-seed globulin is fairly soluble in a ten-per-cent sodium-chloride solution at temperatures above 20° C. At 15° C. it is much less soluble, and at 10° C. it is only very slightly soluble. The solutions of hemp-seed globulin are of a brownish-yellow colour. Although somewhat opaque, the writer was able to use solutions containing as much as two or three per cent of the proteid in a 100-millimetre tube.

* Osborne, *American Chemical Journal*, vol. xiv, p. 673. Chittenden and Mendel, *Journal of Physiology*, vol. xvii, p. 50.

Four independent solutions of hemp-seed globulin were made in a ten-per-cent sodium-chloride solution, and the percentage of globulin in solution was determined by drying at 110° C. The results of the polarimeter observations with these solutions are shown in the following table:

No. of solution.	Ash-free glob. in 100 c. c.	Temperature.	No. of measurements.	Length of tubes.	Mean rot. per 100 mm.	Mean value $(a)_D$.	Probable error.	Remarks.
	Grms.	Deg. C.		Mm.	Deg.	Deg.	Deg.	
1	3·40	25·0	2	100	−1·47	−43·2	±0·5	Solution quite opaque.
2	2·91	26·5	3	100	1·26	43·2	1·0	" " "
4	2·30	24·8	4	100	0·995	43·2	0·5	Less opaque.
1 (diluted)	1·70	24·8	6	100 and 200	0·730	42·9	0·8	
2 "	1·45	26·4	2	100	0·596	41·0	2·0	Eyes fatigued.
2 "	0·97	26·2	4	100 and 200	0·389	40·0	1·1	
4 "	0·92	20·3	6	200	0·365	39·7	0·9	
3	0·80	22·3	9	200 and 300	0·342	43·0	0·9	

With the exception of those for solutions containing 0·97 and 0·92 per cent of globulin, the values obtained for $(a)_D$ agree well within the given limits of error. In general they seem to indicate a decrease in the rotary power of the globulin with the percentage in solution. Assuming such a decrease in the rotary power, by interpolating and extrapolating for each separate solution and averaging the results we obtain $(a)_D = -41{\cdot}6° \pm 0{\cdot}5°$, as the specific rotation of hemp-seed globulin dissolved in a ten-per-cent sodium-chloride solution, each 100 cubic centimetres of the resultant solution containing 1 gramme of the proteid. For purposes of comparison the value

$$(a)_D = -41{\cdot}5°$$

probably represents the specific rotation of hemp-seed globulin in such a solution within at least 1°.

Sodium-sulphate Solutions.

The sodium-sulphate solution used as a solvent was made up to a strength of ten per cent with sodium sulphate dried in a desiccator, but of only five per cent of the anhydrous salt (dried at 110° C.). The hemp-seed globulin was only slightly soluble in this solution at the temperature of the room, and so the globulin solutions were

made at a temperature of about 35° C. To avoid precipitation during the polarimeter observations, the tubes were warmed to about 50° C. and the solution to 35° or 40° C. before filling. Despite the care taken, several sets of observations were spoiled by the precipitation of the globulin.

Only two independent solutions of hemp-seed globulin were made in the sodium-sulphate solution. The results of the polarimeter observations and from drying down at 110° C. were as follows:

No. of solution.	Ash-free globulin in 100 c. c.	Temperature.	No. of measurements.	Length of tubes.	Mean rot. per 100 mm.	Mean value $(\alpha)_D$.	Probable error.
	Grm.	Deg. C.		Mm.	Deg.	Deg.	Deg.
5	1·015	31·8	7	100 and 200	−0·393	−38·7	±0·8
6	0·864	30·0	5	100	0·333	38·6	1·4

These results are quite concordant, and show the specific rotation of hemp-seed globulin in a ten-per-cent (five-per-cent solution of the anhydrous salt) sodium-sulphate solution to be some three degrees less than in a ten-per-cent sodium-chloride solution.

Ammonium-sulphate Solutions.

The hemp-seed globulin was more soluble in a ten-per-cent ammonium-sulphate solution than in the preceding sodium-sulphate solution, but less soluble than in a ten-per-cent sodium-chloride solution.

Two independent solutions of the globulin in a ten-per-cent ammonium-sulphate solution were made, and the following results obtained from them:

No. of solution.	Ash-free globulin in 100 c. c.	Temperature.	No. of measurements.	Length of tubes.	Mean rot. per 100 mm.	Mean value $(\alpha)_D$.	Probable error.
	Grm.	Deg. C.		Mm.	Deg.	Deg.	Deg.
7	1·10	27·5	4	100 and 200	−0·442	-40·0	±0·8
7 (diluted)	0·48	26·5	3	200 " 220	0·205	42·8	5·6
8	1·18	30·4	6	100 " 200	0·456	38·6	1·3

These results, although not very concordant or reliable, are sufficiently so to show that the specific rotation of hemp-seed globulin in an ammonium-sulphate solution lies between the values found respectively for sodium-sulphate and sodium-chloride solutions.

The weighted mean of the above gives for the specific rotation of hemp-seed globulin in a ten-per-cent ammonium-sulphate solution

$$(a)_D = -39.8°.$$

Sodium-chloride-potassium-hydroxide Solution.

The remnants of Solutions 3 and 4 (hemp-seed globulin in a ten-per-cent sodium-chloride solution) were used to form an alkaline solution by adding to them a definite volume of an 8·2-per-cent potassium-hydroxide solution; 100 cubic centimetres of the solution thus formed contained 0·766 gramme of hemp-seed globulin, 9 grammes of sodium chloride, and 0·82 gramme of potassium hydroxide.

Five measurements of the rotation due to this solution gave a mean angle of $-0·371° \pm 0·011°$ for a 100-millimetre tube, the average temperature of the solution being 22·1° C. The specific rotation of the hemp-seed globulin calculated from this is

$$(a)_D = -57·6° \pm 1·4°.$$

After these measurements the solution was heated to 40° C. for fifteen or twenty minutes to insure the complete conversion of the proteid into an alkali-albumin, and, after cooling, its rotary power was again determined.

Four sets of observations with a 100-millimetre tube gave a mean rotation of $-0·412° \pm 0·011°$, the average temperature of the solution being 21·2° C. The specific rotation of the globulin calculated from this is

$$(a)_D = -61·6° \pm 1·4°.$$

This is only 4° greater than the value obtained before warming the solution, which would seemingly indicate that the proteid must have been almost entirely converted into an alkali-albumin before the first determination of the specific rotation was made.

Dilute Potassium-hydroxide Solution.

Two determinations of the amount of moisture in the air-dry hemp-seed globulin were made at different times by drying it at 110° C. The first determination gave a loss of weight of 0·0654 gramme for each gramme of globulin, and the second of 0·0659

gramme, showing that the percentage of moisture remained practically unchanged.

3·3720 grammes of this air-dry globulin were dissolved in an 0·18-per-cent KOH solution, and made up with the same solution to 200 cubic centimetres. The rotary power of this solution of hemp-seed globulin was determined immediately after making it, and again after heating it at 40° or 50° C. for some thirty to forty minutes, with the following results:

	Ash- and water-free globulin in 100 c. c.	Temperature.	No. of measurements.	Length of tube.	Mean rot. per 100 mm.	Mean value $(a)_D$.	Probable error.
	Grm.	Deg. C.		Mm.	Deg.	Deg.	Deg.
Before heating.	1·57	23	4	100	−1·017	−64·7	±1·0
After " .	1·57	25	5	100	0·999	63·6	0·7

The values found for $(a)_D$ agree within the limit of error of observation, and are slightly larger than those obtained for hemp-seed globulin in the last solution. Their agreement would indicate that the action of the alkali in raising the rotary power of the globulin is exerted at once and without the aid of heat, the proteid being presumably changed more or less completely into an alkali-albumin when dissolved in the alkaline solution.

Dilute Sodium-carbonate Solution.

5·6987 grammes of the air-dry hemp-seed globulin were dissolved in a 0·2-per-cent sodium-carbonate solution and then made up with the same sodium-carbonate solution to 240 cubic centimetres. The rotary power of this globulin solution was determined, (1) immediately after making up the solution, (2) six hours after, and (3) after heating it at 40° to 50° C. thirty or forty minutes and cooling. The results of the polarimeter observations were as follows:

	Ash- and water-free globulins in 100 c. c.	Temperature.	No. of measurements.	Length of tubes.	Mean rot. per 100 mm.	Mean value $(a)_D$.	Probable error.
	Grm.	Deg. C.		Mm.	Deg.	Deg.	Deg.
1	2·21	22·0	4	100	−1·072	−48·4	±0·5
2	2·21	22·5	4	100	1·105	49·9	0·4
3	2·21	23·0	4	100	1·200	54·2	0·7

These show a slight increase in the specific rotation during the first six hours, and a marked increase after heating the solution of over 4°. The globulin is evidently not so readily nor so completely transformed by the sodium carbonate as by the stronger caustic alkali.

Dilute Hydrochloric-acid Solution.

3·6906 grammes of the air-dry hemp-seed globulin were dissolved in a 0·26-per-cent hydrochloric-acid solution and made up to 262 cubic centimetres with the same solution. This solution was exceptionally transparent and the most satisfactory for use in the polarimeter of any proteid solution tried by the writer. Its rotary power was determined, (1) two hours after making the solution up, (2) twenty hours after making it up, (3) after heating it at about 40° C. for twenty minutes, and (4) after letting it stand for thirty-four days. The results of the polarimeter observations were as follows:

	Ash- and water-free globulins in 100 c. c.	Temperature.	No. of measurements.	Length of tubes.	Mean rot. per 100 mm.	Mean value $(a)_D$.	Probable error.
	Grm.	Deg. C.		Mm.	Deg.	Deg.	Deg.
1	1·31	24	6	100	−1·120	−85·3	±0·7
2	1·31	20	4	200	1·066	81·2	0·4
3	1·31	21	4	300	1·079	81·9	0·2
4	1·31	24	4	200	1·097	83·6	0·3

The largest value obtained for the specific rotation was within two hours after making the solution up and before heating it. That obtained twenty hours after was some 4° lower, and there was only a slight increase in the specific rotation after the solution had been heated. After the solution had stood for over a month the specific rotation was found to have increased about 1·7°.

III. Flaxseed Globulin.

Preparation and Composition.

On account of the gummy character of the extracts from flaxseed and the consequent difficulty in filtering them, it was not easy to obtain this proteid in large quantities. That used in this investigation was prepared by the following method:

Ordinary flaxseed meal was treated with benzine (petroleum ether) to extract the fat, and then, after drying, was passed through a fine sieve to remove the husk as far as possible. The globulin was extracted from this prepared meal by means of a ten-per-cent sodium-chloride solution and the extract filtered clear. When extracted at 60° C. the extract obtained was richer in globulin but more difficult to filter than when extracted at 20° C. Extracts were obtained at both temperatures. The precipitation of the globulin by cooling was found to be impracticable, even after excessive dilution of the filtrate with water. The extracts were therefore submitted to dialysis for a few days, yielding in every case a fair percentage of well-crystallized globulin. The crystallized precipitate was collected on a filter and washed successively with dilute alcohol, absolute alcohol, and ether, and dried, after the ether had evaporated, in a desiccator over sulphuric acid. The minute crystals thus obtained were perfect isometric octahedra—somewhat smaller than those obtained from the hemp seed.

The reactions of this globulin from flaxseed have been fully given by Osborne,* and differ but little from those found for the hemp-seed globulin. As the result of a number of independent analyses, he found the flaxseed globulin to have the following composition:

Carbon	51·48	Sulphur	0·81
Hydrogen	6·94	Oxygen	22·17
Nitrogen	18·60		100·00

It will be seen by comparison that the composition of this proteid is almost identical with that of the globulin from hemp seed, which it also resembles, as stated, in its reactions, and in the form of its crystals, except that none of the crystals of the flaxseed globulin are of a hexagonal form. Osborne, because of the similarity in their composition and behaviour toward reagents, considers these two globulins—and also those from the castor bean, cotton seed, squash seed, and a number of cereals—as one and the same proteid body.†

* *American Chemical Journal*, vol. xiv, pp. 629, 681.

† *Ibid.*, vol. xiv, p. 687, and vol. xv, p. 24. Also *Rep. Conn. Agric. Exp. Sta. for 1893*, pp. 179, 216, and same for 1895, p. 172.

This body he has named *edestin*, from its occurrence in so many food stuffs.

0·7778 gramme of the flaxseed globulin prepared by the writer, dried at 110° C., yielded, after ignition, a residue of 0·0075 gramme of ash. This would give a percentage of ash of 0·96 per cent.

As the quantity of crystallized flaxseed globulin prepared was not sufficient for an extended investigation of its rotary properties, the specific rotation was determined for only a single solvent, a ten-per-cent sodium-chloride solution being chosen as the most available. A good basis was thus obtained for comparing its rotary power with that of the other proteids studied.

Sodium-chloride Solutions.

These solutions of flaxseed globulin had a rather strong yellow colour. They proved, however, to be fairly transparent to sodium light, so that the writer was able to use in the polarimeter solutions that appeared quite opaque when in a beaker.

Three independent solutions of flaxseed globulin in ten-per-cent sodium chloride were made, and used in the polarimeter. The crystals of the globulin used in the first solution (Solution 13) were somewhat imperfect, having been rounded and broken during the processes of filtering and drying. They were separated by dialysis from a ten-per-cent sodium-chloride extract obtained from the fat-free flaxseed meal by a second extraction at 20° C.* The globulin used in the other two solutions contained none but perfect crystals. Part of it was extracted from the flaxseed meal by a ten-per-cent sodium-chloride solution at 20° C., and the rest was similarly extracted at 60° C. In both cases the globulin was precipitated from the extract by dialysis.

The results of the polarimeter observations and from drying down are tabulated below. With the exception of the first and last, the values found for the specific rotation agree well within the limits of error, although they apparently decrease with the percentage of globu-

* With these were mixed some perfect crystals obtained by cooling from a five-per-cent sodium-chloride extract at 60° C.

lin in solution. The table shows clearly how the error in the mean value of $(a)_D$ is increased when a weak solution is used :

No. of solution.	Ash-free globulin in 100 c. c.	Temperature.	No. of measurements.	Length of tubes.	Mean rot. per 100 mm.	$(a)_D$.	Probable error.
	Grm.	Deg. C.		Mm.	Deg.	Deg.	Deg.
15	1·51	23·7	8	100	−0·572	−38·0	±0·5
13	1·19	25·5	10	100 and 200	0·472	39·7	0·6
14	1·08	24·0	7	100	0·426	39·4	0·9
15 (diluted)	0·94	22·5	8	200 and 300	0·364	38·8	1·2
13 "	0·60	23·0	5	200 and 300	0·225	37·8	1·8
14 "	0·47	23·5	10	200 and 300	0·172	36·7	1·3

The weighted mean of the last column but one gives

$$(a)_D = -38·7°,$$

with a probable error of less than half a degree.

Sodium-chloride-potassium-hydroxide Solution.

A remnant of Solution 15 (diluted), containing 90 cubic centimetres, was made up in a graduated flask to 100 cubic centimetres with an eight-per-cent potassium-hydroxide solution. The solution thus formed contained, in 100 cubic centimetres, 0·85 gramme of flaxseed globulin, 9 grammes of sodium chloride, and 0·8 gramme of potassium hydroxide.

The colour of the solution was changed by the potassium hydroxide from a bright-yellow to an orange tint, which made it more difficult to measure the rotation, even with a 100-millimetre tube. As a result of four measurements with a 100-millimetre tube at the temperature of 23° C., the rotation of the plane of polarization was found to be $-0·464° \pm 0·013$. This would give as the specific rotation of flaxseed globulin in such an alkaline solution,

$$(a)_D = -54·5° \pm 1·5°$$

IV. Brazil-nut Globulin.

Preparation and Composition.

The globulin obtained from the Brazil nut was chosen for investigation because it differed somewhat in its chemical composition and reactions from the two previously studied. These differences are regarded by Osborne as sufficient to warrant its being classed as

a distinct proteid body.* The preparations used in this investigation were obtained by the following methods:

A fine white meal was prepared from the meat of the Brazil nut by grating it, extracting the oil with benzine, and then sifting it through a fine sieve. Two methods were employed to obtain the globulin. By the first it was extracted from the prepared meal with distilled water at 60° C. and then precipitated by cooling. This precipitate was collected on a filter and washed successively with dilute alcohol, absolute alcohol, and ether, and dried, after the evaporation of the ether, over sulphuric acid. By the second method the globulin was extracted from the prepared meal with a ten-per-cent sodium-chloride solution at 20° C., and the extract submitted to dialysis for a few days. The resulting precipitate was collected on a filter and washed and dried in the same manner as that obtained by cooling from the water extract. The second method gave the largest yield of globulin, and was on the whole found to be the more satisfactory, although the preparation was not as well crystallized as that from the water extract. In neither case were the globulin crystals as perfect as those obtained from the flaxseed and hemp seed. The Brazil-nut globulin crystals varied in form from a thin hexagonal plate, through more or less rounded forms, to a perfect spheroid. The most common form, and in fact the only one found in the globulin precipitated by cooling, was a thick hexagonal plate with every other angle rounded off. In bulk the crystallized globulin appeared as a very fine, pure-white powder.

This globulin from the Brazil nut has been analyzed by Sacchse, Ritthausen, and others.† The following analyses are given by Osborne:

	Crystals.	Spheroids.	Spheroids.
Carbon	52·18	52·35	52·16
Hydrogen	6·92	6·96	6·98
Nitrogen	18·30	18·16	18·32
Sulphur	1·06	1·12	1·07
Oxygen	21·54	21·41	21·47
Totals	100·00	100·00	100·00

* *American Chemical Journal*, vol. xiv, p. 687. † *Ibid.*, p. 669.

The writer found the percentage of ash to be 0·42 per cent in the globulin preparation obtained by cooling a hot aqueous extract of the Brazil-nut meal.

Sodium-chloride Solutions.

The globulin from the Brazil nut was quite soluble in a ten-per-cent sodium-chloride solution, forming a rather dense opaque solution—so opaque to sodium light that it was difficult to measure its specific rotation, even with solutions containing from 0·5 to 1·0 per cent of the proteid. The opacity of its solutions and the limited quantities in which this globulin could be prepared prevented an extensive investigation of its rotary properties. As with the flaxseed globulin, the specific rotation of the Brazil-nut globulin was only determined for solutions with sodium chloride.

Three solutions of this globulin in a ten-per-cent sodium-chloride solution were prepared. The crystallized globulin used in solutions numbered 17 and 19 was obtained by cooling a hot (60° C.) water extract of fat-free Brazil-nut meal. That used in Solution 18 was obtained by dialysis from a cold (20° C.) ten-per-cent sodium-chloride extract of the Brazil-nut meal. This last solution was of a whitish colour and much more opaque to sodium light than the others. After repeated dilution a measurement of its rotary power was finally effected, but the result was so poor that no attempt was made to determine the small correction for the ash present.

The results of drying the solutions down at 110° C. and of the polarimeter observations are shown in the following table:

No. of solution.	Globulin in 100 c. c.	Temperature.	No. of measurements.	Length of tube.	Mean rot. per 100 mm.	$(\alpha)_D$.	Probable error.	Remarks.
	Grm.	Deg. C.		Mm.	Deg.	Deg.	Deg.	
17	2·13	22·3	6	100	−0·866	−40·75	±0·4	
17 (diluted)	0·92	20·8	12	100 and 200	0·366	39·9	1·9	
19	0·92	23·3	6	100	0·390	42·5	1·3	
18	0·77	22·3	2	100	0·285	37·1	2·4	Not corrected for ash.
17 (rediluted)	0·55	20·3	7	200 and 300	0·184	33·5	1·5	Unreliable; trace of ether.
19 (diluted)	0·45	22·7	9	200	0·173	38·1	0·9	

The values found for $(a)_D$ are not very concordant or accurate, but are the best that the writer could obtain with such opaque solutions. Omitting the value $-33{\cdot}5°$, which was somewhat doubtful, and the value $37{\cdot}1°$, which was not corrected for ash, the weighted mean of these is

$$(a)_D = -40{\cdot}3°.$$

Sodium-chloride-potassium-hydroxide Solution.—When potassium hydroxide was added to a sodium-chloride solution of Brazil-nut globulin the resulting solution had to be made much more strongly alkaline to prevent precipitation than with solutions of either flaxseed or hemp-seed globulin.

Sixty-nine cubic centimetres of Solution 17, diluted, were made up with an 8·2-per-cent potassium-hydroxide solution to 100 cubic centimetres. The resulting solution contained, in 100 cubic centimetres, 0·633 gramme of ash-free globulin, 6·90 grammes of sodium chloride, and 2·55 grammes of potassium hydroxide.

The rotary power of this solution was determined within three hours after making up, and again after heating to 50° C. for twenty minutes, with the following results:

	Globulin in 100 c. c.	No. of measurements.	Length of tube.	Mean rot. per 100 mm.	$(a)_D$.	Probable error.	
	Grm.		Mm.	Deg.	Deg.	Deg.	
Before heating...	0·63	5	100	−0·370	−58·5	±1·2	Temperature about 20° C.
After " ...	0·63	5	100	0·364	57·5	1·3	

The value of $(a)_D$ obtained after warming the solution agrees, within the limits of error, with that obtained before warming it. It is therefore obvious that the full action of the alkali is exerted without the aid of heat.*

V. Summary and Conclusions.

The specific rotation obtained from ten-per-cent sodium-chloride solutions, containing about one per cent of proteid matter, were as follows:

* In summarizing, the values of $(a)_D$ are given to the nearest 0·5°.

Hemp-seed globulin ..... $(\alpha)_D = -41{\cdot}5°$
Brazil-nut globulin ..... $(\alpha)_D = -40{\cdot}5°$
Flaxseed globulin ..... $(\alpha)_D = -38{\cdot}5°$

The differences in the rotary powers of these substances are not very large or unexpected when we consider the slight differences in their chemical composition and behaviour, and in their content of ash.

Effect of Dilution.—The observations made with sodium-chloride solutions of these globulins showed in general a decrease in the rotary power with the percentage of proteid in solution, although the observations were not sufficiently accurate to determine the rate of this decrease.

Effect of Temperature.—As already stated, the changes in the specific rotation with the temperature were apparently very slight, and no attempt was made to determine them.

Effect of Solvent.—The following results obtained with the globulin from hemp seed show that the salt solution used as a solvent was not without influence on its rotary power:

Solvent.	Value of $(\alpha)_D$.
10-per-cent sodium-chloride solution	$-41{\cdot}5°$
" ammonium-sulphate solution	$-40{\cdot}0°$
" sodium-sulphate Equals 5-per-cent anhydrous salt solution	$-38{\cdot}5°$

Alkali-albumins.—The differences in the specific rotations of the globulins studied have already been noted. Their alkali-albumins also differ in their rotary powers in the same order, as the following table shows:

Proteid.	Per cent proteid.	Per cent NaCl.	Per cent KOH.	Value $(\alpha)_D$.
Hemp-seed globulin	0·75	9·0	0·8	$-61{\cdot}7°$ *
Brazil-nut "	0·65	6·9	2·55	$-58{\cdot}0°$ †
Flaxseed "	0·85	9·0	0·8	$-54{\cdot}5°$

Alkaline and Acid Solutions.—The globulin from hemp seed was the only one whose rotary power was investigated in dilute alkaline and acid solutions. In general, as with other proteids, the effect of

* This was the value of $(\alpha)_D$ after heating the solution. Heating increased it.
† Heating this solution did not materially affect the value of (α).

the alkali or acid was to increase the specific rotation from fifty to one hundred per cent, this increase being greatest in the acid solutions. Except in the sodium-carbonate solution, the increase in the rotary power took place almost immediately, and heating the solution did not materially alter its value. When dissolved in 0·2-per-cent hydrochloric acid there was at first a large increase and then a slight decrease in the rotary power of the proteid. The following are the results obtained with alkali and acid solutions:

Solvent.	Per cent proteid.	$(\alpha)_D$.
0·18 per cent potassium hydroxide	1·5	−64·5° within an hour. −63·5° after heating.
0·20 per cent sodium carbonate	2·2	−48·5° within an hour. −50·0° after 6 hours. −54·0° after heating.
0·26 per cent hydrochloric acid	1·3	−85·5° within 2 hours. −81·0° after 20 hours. −82·0° after heating. −83·5° after 34 days.

It is worth noting that the specific rotations of the animal and vegetable globulins, so far as known, lie between −38° and −48°, or perhaps within still closer limits. Besides those studied by the writer, the specific rotation of serum globulin is −48°, that of fibrinogen lies between −45° and −50°, and we have good reason for believing that that of egg globulin is about −40°.

In conclusion, the writer desires to acknowledge his indebtedness to Prof. R. H. Chittenden and Dr. L. B. Mendel, of the Sheffield Scientific School, for assistance and suggestions in the preparation of the proteids and their solutions; the success of this investigation is due in a large measure to their able and hearty co-operation. And also to Prof. C. S. Hastings, of the same institution, in whose laboratory the polarimeter observations were all made.

A CHEMICAL STUDY OF THE SECRETION OF THE ANAL GLANDS OF MEPHITIS MEPHITICA (COMMON SKUNK), WITH REMARKS ON THE PHYSIOLOGICAL PROPERTIES OF THIS SECRETION.

By THOMAS B. ALDRICH, Ph. D.

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The subfamily *Mephitinæ*, to which the skunk belongs, is represented in the United States by three genera: *Spilogale*, *Conepatus*, and *Mephitis*. *Mephitis* includes all of the large common skunks of the United States except the so-called "badger" skunks along our Southern boundary, which belong to the genus *Conepatus*. Three species are referred to *Mephitis*: *M. macrura*, *M. putorius*, and *M. mephitica*. The latter species is found from Hudson's Bay to Guatemala, but is more frequently met with in the Northern and Eastern States.

Before considering the secretion itself it will be well to describe briefly the anal glands or pouches, common not only to the subfamily *Mephitinæ*, but also to the family *Mustilidæ*. The two pouches in which is secreted the fluid emitting the offensive odour that has made the skunk notorious are situated on either side of the rectum close to the anus. They are oval in shape, the longer diameter from an inch to an inch and a half, surrounded by a muscular envelope and opening into the rectum just inside the sphincter by single ducts which terminate at the top of prominent nipplelike papillæ. The papillæ are situated at the bottom of recesses, and when not protruded are nearly concealed from view by folds of the mucous membrane. By gentle pressure the fluid in the sac may be forced out through the duct in a very fine stream.

There is still prevalent an erroneous idea that the secretion is identical with the urine or is in some way connected with it. That

there is no connection whatever between the sacs and the genito-urinary passages has been shown by Wyman * and Chatin.† The latter especially has made a thorough anatomical study of the anal glands of *Conepatus mapurito*. His observations may be summed up as follows: Below the muscular layer—that is to say, within the general muscular envelope—is found the follicular or glandular portion proper of the organ; it is not regularly distributed around the central reservoir, as in most carnivora, but occupies only a limited portion of the surface of the receptacle. The follicles are rather large and of a reddish-brown colour; their numerous well-developed *culs-de-sac* measure on an average 0·55 millimetre in diameter, and are variously rounded, ovoidal, club-shaped, etc. The reservoir is lined with a comparatively thick whitish tunic composed of dense laminated tissue and elastic fibres.

Dr. L. F. Barker, Associate in Anatomy in the Johns Hopkins University, has kindly furnished me with the following analysis of a series of sections from tissues obtained from the common skunk, which were prepared for me in the Pathological Laboratory by Dr. George W. Dobbin:

"The sections go through the wall of the sac in different parts, and have been stained in hæmatoxylin and eosin. The wall consists of an internal mucous membrane, a submucous coat, a muscular coat of striped fibres, and an external fibrous coat. The masses of glands are situated in the submucosa. Leading from the reservoir to the outside is a duct lined by stratified epithelium which opens at the apex of the papilla or nipple.

"*Tunica Externa*.—This is thin, consisting of fibrous tissue poor in cells. A few fat-cells are present in places, and large veins are met with in transverse and longitudinal section.

"*Tunica Muscularis*.—The muscular coat varies considerably in thickness, being about four millimetres in its thickest part and one millimetre in its thinnest part. It consists of bundles of long striated muscle fibres, which in places run all in one direction; at other parts of

* *Proc. Boston Soc. of Nat. Hist.*, 1844, i, p. 110.

† *Ann. des Sciences nat.* [5], 1874, xix, p. 100.

the wall the fibres of the outer part run at right angles to those of the inner. The individual muscle fibres are of large size, are exquisitely cross-striated, and show spindle-shaped nuclei in the periphery of the fibre quite like those of human muscle. The internal perimysium carrying blood vessels is everywhere delicate.

"*Tela Submucosa.*—This consists of a rather loose meshwork of fibrous tissue intervening between the mucous membrane and the muscle. In it are situated the masses of glands. These are not evenly distributed throughout the wall of the sac, but occur in masses in various parts, being particularly abundant at the fundus of the reservoir. In the submucosa, arteries of medium size and larger veins are situated; and near the glands, between them and the muscle, bundles of medullated nerve fibres of considerable size are to be made out.

"The masses of glands themselves show gland tubes cut in various directions transversely, longitudinally, and obliquely. They appear to be coil glands, although they may branch. The amount of connective tissue between the coils is insignificant, there being just enough to carry a rich supply of blood capillaries. Sections of the glands show the tubules to be lined by cubical epithelium; the nuclei of the cells are situated rather nearer the proximal than the distal ends of the cells. They are round, or slightly oval, and stain intensely in hæmatoxylin. The distal portion of the protoplasm stains rather more feebly than the proximal portion, the latter taking a deeper tint in hæmatoxylin and tending to be slightly granular. The lumen of the tubule varies in different parts. In places it is wide, in other places reduced to a narrow slit. Between the proximal ends of the epithelial cells and the basement membrane long, spindle-shaped nuclei can be seen whose long axis is, as a rule, parallel to the course of the gland tubule. These are evidently the nuclei of smooth muscle fibres. The basement membrane itself appears delicate and homogeneous. Here and there within a mass of tubules an artery or vein of considerable size may be met with.

"*Tunica Mucosa.*—This consists of an epithelial covering and a tunica propria. The epithelial covering consists of several layers of

epithelial cells, the deepest layers tending to be cubical, the more distant layers to be polyhedral in shape. As a rule, the layer is from four to five cells deep. All of the cells distal to the proximal row show a network within the cell protoplasm, the meshes of which are filled up by clear hyaline droplets. The appearances in these cells resemble very closely those to be seen in the cells of sebaceous glands.

"The superficial cells of the epithelial layer may be nucleated or non-nucleated, and in this layer the protoplasm contains large numbers of irregular masses and particles which stain deeply in hæmatoxylin, but metachromatically, taking a reddish-brown tint. These particles resemble closely those seen in the granular cells at the periphery of the stratum germinativum (Malpighii) in human skin (kerato-hyalin—Waldeyer). In places on the surface or desquamated from it can be seen a layer of cells which hang together, which are almost entirely devoid of chromatic substance and look like pale vesicles united edge to edge.

"The tunica propria of the mucous membrane is a little less than 1 millimetre in thickness, and consists of bundles of white fibres which for the most part run parallel to one another, taking a somewhat wavy course. Nuclei of connective-tissue corpuscles are visible between the bundles, and small blood vessels are also present in this layer. There are no distinct papillæ to be made out in the wall of the reservoir, although they are present in the duct leading from the reservoir through the papilla to its apex.

"*The Papilla or Nipple.*—In transverse section this is seen to be lined by stratified epithelium resting upon a distinctly papillated tunica propria. The papilla is rich in voluntary muscle fibres, the mucous membrane lining it being surrounded by two rows of bundles of fibres which run parallel to the long axis of the duct. External to these is a tolerably thick layer of muscle fibres which run for the most part circularly, although some of them run obliquely. Nowhere in the papilla or in the reservoir can smooth muscle be made out except, as before described, in the structure of the glands themselves. Outside the voluntary muscle of the papilla is a firm fibrous

coat. In the connective tissue of the papilla and here and there among the epithelial cells branched pigment cells are visible.

"The histologist must be struck by the resemblance of the structure of the glands in the wall of the reservoir to that of the ordinary sudoriferous glands of the skin. Indeed, it is not inconceivable that these glands of the skunk may represent curiously modified sweat glands. As to the nature of the reservoir with its curious epithelial lining, it is difficult to decide. If the glands in the wall of the reservoir of the skunk should turn out to be homologous with sweat glands, the relation of the reservoir to sweat pores will have to be considered. In this connection, the curious character of the epithelium cells lining the reservoir, reminding one somewhat of the structure seen in sebaceous glands, must not be forgotten; and any attempt to explain the origin and the character of the secretion should consider not only the products of the glands in the submucosa, but also the part, if any, played in its formation by the epithelium of the reservoir itself."

The exceedingly disagreeable odour possessed by the secretion of the *Mephitinæ*,* and the difficulty attending its collection in sufficient quantity for an exhaustive study have deterred investigators in the past from studying this most singular and interesting fluid.

If one were bold enough to start the investigation, objections were invariably raised, and thus all further research was stopped. At least this was Loew's† experience, who says: "On an expedition through Texas in 1872 I had frequent opportunity to collect a sufficient quantity of this secretion to establish its chemical constitution, but all my companions protested against it, declaring the odour which clung to me to be unbearable. On my return to New York city I started a few chemical tests, with the little I had collected, when the whole college rose in revolt, shouting, 'A skunk, a skunk

* I am informed by Dr. C. Hart Merriam, Chief of the Division of Mammalogy and Ornithology, Washington, D. C., that the secretion of some other members of the family *Mustilidæ* is, if possible, more offensive than that of the skunk. The secretion of the mink is not so diffusible or penetrating, but it is more nauseating.

† *Aerztliches Intelligenzblatt von München*, June 10, 1879, p. 252.

is here!' I had to abandon the investigation." * This, no doubt, has been the experience of others who have attempted to study either the secretion or the glands. I have been more fortunate than my predecessors in being surrounded by those who, for the cause of science, would endure even the odour of a skunk in close proximity. Had it not been for this forbearance on their part, I should undoubtedly have suffered the fate of others.

Lassaigne,† Swarts ‡ (in Wöhler's Laboratory), and Loew # have all made the secretion of the skunk the subject of chemical research, but it is to be regretted that the origin of the fluid investigated by the two former was unknown. ‖

Lassaigne separated the secretion into a heavy and a light oil.

Swarts found it to consist of a colourless portion boiling between 105° and 110° C., and a heavy yellow oil boiling between 195° and 200° C. In the residue he recognised a nitrogenous basic body which, from its volatility, he concluded to be either methylamine or ethylamine.

According to Loew, the secretion consists of at least two bodies, one of which is richer in sulphur than the other, possesses properties similar to the oil obtained from garlic (*Knoblauchöl*), gives with the metals salts, and is violently attacked by concentrated nitric acid; the other oil contains nitrogen and gives rise, under certain conditions, to a base which smells like trimethylamine.

The results of these investigators amount to very little from a chemical point of view. Nothing definite as to the real chemical nature of this secretion has hitherto been determined.

The material for this investigation was obtained principally from the State of Maine; a small quantity, however, came from Long Island. With one exception, it came in the sacs, which were emptied as soon as received. The quantity of fluid in the sacs varies

* From a letter to Prof. Abel.

† Cited by Swarts.

‡ Liebig's *Annalen*, 1862, cxxvii, p. 266.

Op. cit., p. 252.

‖ It is very probable that the secretion of the other members of the subfamily *Mephitinæ* differs very little from that of *M. mephitica*.

greatly; some were found empty, others contained as much as 5 cubic centimetres, or even more if they were very much distended. I presume the age, vigour, and size of the animal, also the method employed in capturing, will explain this variation.

The length of time that elapsed before the sacs reached the laboratory varied from twelve hours to several days. In those cases where no especial care had been taken to prevent decomposition the sacs still gave no evidence of change. It is probable that the secretion preserves from immediate decomposition not only the tissue in contact with it, but also that in the immediate neighbourhood. No differences that could be attributed to decomposition were observed in any case, the odour and colour of the secretion from the different sacs being approximately the same.

Physical Properties of the Fluid.

Obtained from the sacs a few hours after the death of the animal, the secretion is a clear, limpid fluid, of a golden-yellow or light-amber colour, of a characteristic, penetrating, and most powerful odour, and having a specific gravity, at ordinary temperature, less than water (0·939).

The fluid filters readily, sometimes shows a decided greenish cast, and in the evening is said to be slightly luminous; but I myself have not been able to verify the last observation. After removal from the sac it turns gradually darker in colour, probably on account of a slight oxidation. It remains fluid at a temperature of −13° C.

There is generally present in the sacs to the extent of one gramme or more a yellowish amorphous body, having, as far as I have tested, an alkaline reaction, and giving the proteid tests. I take this substance to consist in large part of the *débris* of desquamated epithelial cells from the inner surface of the sac. Whether any chemical function is to be ascribed to this *débris* I have not yet been able to determine. Prismatic crystals have been observed when a little of the fluid has remained some time in contact with the amorphous body referred to. The secretion standing in a bottle often deposits a white flocculent substance.

Chemical Properties of the Fluid.

The reaction of the fluid I found to be neutral; others have found it intensely acid;* but I can not reconcile this statement with the character of the chemical bodies found in the secretion. These substances without doubt belong to groups that have as one of their characteristic properties a neutral reaction.

The vapours of the fluid are highly inflammable and burn with a luminous flame, giving off sulphur dioxide.

Absolute alcohol, ether, and chloroform dissolve the fluid readily. Concentrated sulphuric acid dissolves it, but not without producing some changes, as the colour changes to a deeper hue. The body is thrown out of the alcoholic and concentrated sulphuric-acid solutions on dilution with water, in which it is practically insoluble. The odour in these solutions is but little changed.

A fifty-per-cent solution of sodium or potassium hydroxide dissolves the fluid partially, the peculiar odour disappearing almost entirely. From this solution the oil is liberated on dilution with much water; the addition of dilute sulphuric acid brings back the original odour. The same facts may be shown in the following way:

A strip of filter paper moistened with an aqueous solution of lead acetate and suspended over the natural secretion takes on a bright-yellow colour; held over the secretion that has been dissolved in a fifty-per-cent solution of potassium hydroxide, no change is noticed, though it may be in time somewhat darkened in colour; but if this potassium-hydroxide solution is acidified by the addition of sulphuric acid, the bright-yellow colour is again seen in the filter paper held over it. Paper moistened with copper sulphate is acted on in the same way, only that the colour is not so pronounced.

The dilute sulphuric-acid solution, or the aqueous solution, for it is soluble to a slight extent in water, reacts toward an iodine solution similarly to the corresponding solutions of alkyl-sulphides.† Bromine acts like iodine.

* Merriam. Mammals of the Adirondacks, p. 76.

† Abel. *Zeit. f. physiol. Chem.*, xx, pp. 271–273.

An alcoholic solution of the secretion gives with alcoholic solutions of lead acetate, mercuric cyanide, mercuric, platinic, or auric chloride, for the most part amorphous precipitates. The lead compound is yellow in colour, the mercuric compounds are white, and the platinic and gold chloride compounds are orange. Mercuric oxide reacts either directly in the fluid, or in an ethereal solution of the same very energetically, as does also concentrated nitric acid; both form crystalline compounds. An alcoholic solution of ferric chloride gives with the corresponding solution of the secretion a light-green colour.

These physical and chemical properties just referred to warrant the assertion that one or more mercaptans, or thioalcohols, are present in the secretion.

Fractional Separation of the Secretion.

Being convinced of the presence of mercaptans in the secretion, fractional distillation suggested itself as the best method to use in their separation. Accordingly, the filtered secretion was distilled from a suitable bath. With the mercury completely in vapour, a clear, colourless fluid constituting about half of the secretion, distilled over between 100° and 130° C. The bath temperature was generally about 130°, never over 150° C. The temperature then fell, though the bath temperature was increased to 180° C. As there was no evidence of decomposition, it is natural to suppose that these bodies are in the original secretion, and not decomposition products. By this method we are enabled to separate the secretion into two sharply defined and nearly equal portions:

1. The more volatile portion, boiling between 100° and 130° C., and which for convenience we will designate A.

2. The less volatile portion, left after distilling off A, and which we will call B.

An apparatus so arranged as to detect any gas that might be given off under 100° C. gave a negative result. The three lower mercaptans—methyl (B. P.), 6°; ethyl (B. P.), 36°; normal propyl (B. P.), 67°; and iso-propyl (B. P.), 57° to 60° C.—were thus

shown to be, in all probability, absent. We are therefore compelled to seek our mercaptans among those with boiling point near or over 100° and still under 130° C.

Fraction A.

A is a mobile colourless fluid lighter than water and having a very unpleasant penetrating odour, scarcely to be distinguished from that of the original secretion. It gives, without exception, all of the reactions of the original secretion.

After drying over calcium chloride for twenty-four hours it was distilled fractionally. Three fractions were obtained:

1. α, boiling between 100° and 110° C. and constituting about one half of A.

2. β, boiling between 110° and 120° and constituting one fourth of A.

3. γ, left in the bulb after distilling off the two former and constituting one fourth of A.

These three fractions—α, β, and γ—give the mercaptan reactions; that is, they unite with mercuric oxide, mercuric cyanide, and lead acetate to form compounds characteristic of the mercaptans. β was found to contain less sulphur than α, a result to be expected if a higher mercaptan were present. For the purpose of identifying the mercaptan in fraction α, several analyses were made.

Sulphur Analysis.

A quantitative determination, according to Carius, of the percentage of sulphur gave the following results:

I. 0·1535 gramme gave 0·3955 gramme $BaSO_4$.
II. 0·2453 " " 0·6250 " "

	Calculated for C_4H_9SH.	Found.	
		I.	II.
S	35·55 per cent.	35·37 per cent.	34·98 per cent.

In each analysis the sulphur is found too low. This may be readily explained by assuming the presence in small quantity of either amyl mercaptan, the sulphur contents of which are about thirty-three per cent, or of one of the higher sulphides.

Lead Butyl Mercaptide, $(C_4H_9S)_2Pb$.

This compound was prepared by adding an excess of a saturated alcoholic solution of lead acetate to the alcoholic solution of the fraction *a*. It was obtained in beautiful glistening, yellow, rhombic plates, which, recrystallized from alcohol, washed with alcohol and ether, and dried *in vacuo* over sulphuric acid, gave the melting point 85° to 90° C. The compound is very unstable, decomposing readily into the corresponding mercaptan and sulphide of the metal. Possibly this instability may explain the great range of its melting point. Dilute hydrochloric acid as well as dilute sulphuric acid decomposes it, and more readily on heating. Prepared as given above, carbon, hydrogen, and lead determinations were made:

I. 0·2255 gramme gave 0·2106 gramme CO_2 and 0·0802 gramme H_2O.*
II. 0·2453 " " 0·2277 " " " 0·0858 " " *
I. 0·2033 " " 0·1584 " $PbSO_4 = 53·22$ per cent of Pb.
II. 0·1255 " " 0·0974 " " $= 53·02$ " "

	Calculated for $(C_4H_9S)_2Pb$.	Found. I.	II.
C	25·00	25·45*	25·31*
H	4·68	3·95*	3·89*
Pb	53·64	53·22	53·02

Mercuric Chloride Butyl Mercaptide, $(C_4H_9S.HgCl)$.

This body is precipitated as an amorphous compound, though inclined to crystallize, when alcoholic solutions of mercuric chloride and of fraction *a* are brought together. It is practically insoluble in cold alcohol, sparingly soluble in hot, from which it crystallizes in glistening white plates. Recrystallized from hot alcohol three times and dried *in vacuo* over sulphuric acid, it decomposes partially without melting when heated to 185° C. This compound, being stable in the air and having been recrystallized a number of times, gave results which compare more favourably with the theoretical figures.

I. 0·2746 gramme gave 0·1963 gramme of HgS = 61·62 per cent. of Hg.
II. 0·1658 " " 0·1192 " " $= 61·94$ " "

	Calculated for $C_4H_9S.HgCl$.	Found. I.	II.
Hg	61·63	61·62	61·94

* These results were obtained from the analyses of the lead mercaptide prepared as described on page 335

Mercuric Butyl Mercaptide, $(C_4H_9S)_2Hg$.

When mercuric oxide is added to either *a* or its ethereal solution energetic reaction takes place, the oxide disappears, and a white, for the most part amorphous body, somewhat soluble in ether, is deposited. It can be crystallized from its ethereal solution and obtained in white rhombic plates. After having been dried *in vacuo* over sulphuric acid, it melted sharply at 94° to 95° C. to a clear colourless fluid. On cooling it solidified to a mass of crystals.

This compound, like most of the metallic derivatives of the mercaptans, decomposes readily; even during the process of recrystallization from ether, partial decomposition takes place; it turns gradually dark when kept for some time.

0·1108 gramme gave 0·1053 gramme CO_2 and 0·0386 gramme H_2O.

	Calculated for $(C_4H_9S)_2Hg$.	Found.
C	25·40	25·90
H	4·76	3·88

The difficulty of making a carbon determination directly with the fraction *a* will be understood when it is known that a portion of the carbon is deposited in the glass bulb before the entire fluid has volatilized. It was thus found impossible to burn all of the carbon.

The results of these analyses are sufficiently near the theoretical figures when considered together with the boiling point of *a* to convince, I think, the most sceptical that the greater part of this fraction contains one of the butyl mercaptans.

Primary normal butyl mercaptan is given as boiling at 97° C.* The boiling of *a* between 100° and 110° C. could be easily explained if we assume the presence in small quantity of some higher boiling body. This assumption would also explain in general the analytical results. I am inclined to believe in the presence of a higher mercaptan (say amyl mercaptan) rather than a sulphide. The other fraction of A will be taken up at some future time.

After the above analyses had been made I was fortunate enough

* When the mercury is completely in the vapour it will be found to boil a few degrees above 97°.

to obtain a larger quantity of the secretion. This I treated in the manner already described as far as the separation of A and B is concerned. From this point A was not at first subjected to fractional distillation, but shaken up with a fifty-per-cent solution of potassium hydroxide in order to dissolve out the mercaptans, and then repeatedly shaken out with ether to remove any body not soluble in alkali. The results show, however, that when the first distillation is conducted carefully, A is practically free from any bodies outside of the mercaptans, there being very little oil or residue left after the ethereal solution is allowed to evaporate. Dilute sulphuric acid was then added to the alkaline solution, keeping it in an ice mixture, and the mercaptans, free of any possible sulphides, collected with ether. This ethereal solution was dried over fused calcium chloride for twenty-four hours and then, after filtration, subjected to fractional distillation; the same fractions were obtained as before; the boiling point and quantity of each fraction remained practically the same.

For comparison, the mercuric mercaptide obtained by adding mercuric oxide to an ethereal solution of α was made and recrystallized from ether. Dried in the usual manner, the melting point was found to be the same as the mercaptide previously described—94° to 95° C.

The lead mercaptide was also prepared in the usual manner and combustion analyses made. The results of these analyses have been already given under Lead Butyl Mercaptide, page 333.

Synthetical Formation of the Higher Mercaptans.

Several attempts were made to prepare synthetically a mercaptan having an odour and other properties similar to the fraction α or β.

The method employed was that used by Klason (*Berichte*, xx, p. 3904). The alcohols used were iso-butyl alcohol and iso-amyl alcohol. The results obtained with iso-butyl alcohol were not satisfactory; there appeared to be decomposition products formed when the alcohol was allowed to drop into the concentrated, and fuming sulphuric acid. The results obtained with iso-amyl alcohol, how-

ever, were very satisfactory. About fifty grammes of pure iso-amyl mercaptan boiling between 115° and 120° C. were obtained which resembled in all outward appearances the fractions α and β already described, and its odour in concentration or dilution was hardly to be distinguished from that of these fractions or from that of the original secretion. It gave the mercaptan reactions, and could not by any of these reactions be distinguished from the fractions α and β of A.

I intend in the near future to prepare the normal mercaptans of these higher alcohols and compare them with the fractions of A.

Portion of the Secretion called B.

B has a neutral reaction, a dark-red colour, and an aromatic and less penetrating odour than either A or the secretion. The original colour of the secretion can be nearly brought back by adding a volume of alcohol or ether to B equal to A. This seems to indicate that very little decomposition had taken place in distilling off A.

B differs from A in containing nitrogen and in not reacting with mercuric oxide, mercuric cyanide, or lead acetate; it resembles A in giving all the other tests referred to under the secretion. I therefore infer the absence of mercaptans from B. This inference was proved to be correct, for upon shaking B with a fifty-per-cent solution of potassium hydroxide, and subsequently acidifying and shaking out with ether, a trace of an oil was obtained which failed to give the mercaptan tests.

The fact that B contains no mercaptans and still contains a large amount of sulphur, that it is insoluble in a fifty-per-cent solution of potassium hydroxide, that it reacts with mercuric chloride, also with iodine and bromine, and that it is violently attacked by concentrated nitric acid, justify me in predicting the presence of alkyl sulphides, or bodies closely related to them, in this portion of the secretion. I am inclined to believe, from certain tests that I have made, that these sulphur bodies are combined or mixed with some basic body containing nitrogen.

B, together with a basic body isolated from it, will form the subject of another article.

The Odour of the Secretion.

It is hardly necessary to say that the odour of the secretion is exceedingly persistent, diffusible, and penetrating.

The essential principle in the secretion has been attributed to various substances, allyl sulphide and the lower mercaptans * being the ones usually suspected.

The great diffusibility and the penetrating property of the secretion is due to fraction A—that is, to the mercaptans; the permanency of the odour of the secretion is attributable to B, which tends to retain A and at the same time to modify its odour.

Amount of the Secretion that may be recognised by the Sense of Smell.

In order to test the sensitiveness of the olfactory nerves toward the fraction *a* and thus establish its identity with the more volatile and objectionable portion of the secretion, and also its relation to the mercaptans, the following experiments were performed:

A known weight of *a* was dissolved in 500 cubic centimetres of absolute alcohol. To 10 cubic centimetres of this solution another known quantity of alcohol was added, and from this last solution 2 or 3 cubic centimetres were taken, placed in a bottle similar to one used for washing gases, and blown out into a room by means of a bellows. Room capacity, 696 cubic metres.

The weighing took place in another building far from the room used, and the person who manipulated the bellows and mixed the portion of *a* with the air in the room had not been in the building where the secretion was kept and the weighings made. The mixing was continued for at least ten minutes by using large pieces of stiff paper as fans. At a given signal persons who had not previously been near the substance entered the room.

First Trial.—0·1 of a milligramme of *a* was mixed with the air of the room. The odour was very apparent to all on entering. My power to detect the mercaptans had, however, been so blunted by previous contact

* M. v. Nencki u. N. Sieber, *Sitzb. d. kais. Akad. in Wien*, Mathem. Classe, Mai, 1891, xcviii Abth. II. b., S. 1; Swarts, Leibig's *Annalen*, 1862, cxxiii, S. 266.

with the substance that I was unable to detect in this dilution any odour whatever, although I could still detect mere traces of other odoriferous bodies. 0·1 of a milligramme diluted with 696 cubic metres of air allows $\frac{1}{6900000}$ milligramme to each cubic centimetre.

Second Trial.—0·01 of a milligramme of *a* was distributed through the same room several days after the first trial and after the room had been thoroughly ventilated. The odour was faintly apparent to all on entering, but was not noticeable after they had remained even a short time in the room. After they had freed the nostrils of the odour in the open air they could again detect it on re-entering the room. In this trial the limit of dilution was approximately reached. In this dilution we have in every cubic centimetre $\frac{1}{69000000}$ of a milligramme. Granting that 100 cubic centimetres of this air are necessary to make the odour evident in this dilution, we are able to recognise approximately $\frac{1}{6900000}$ of a milligramme of *a* and about four times that quantity of the secretion.

E. Fisher * and Penzoldt have found by similar experiments that one is able to recognise $\frac{1}{48000000}$ of a milligramme of ethyl mercaptan. These results prove the nose to be by far the most delicate testing apparatus known, for the smallest quantity of sodium that can be detected by the spectroscope is nearly two hundred and fifty times larger † than the amount required of ethyl mercaptan to rouse the sense of smell.

The similarity of ethyl mercaptan and the fraction *a* is very striking in respect to diffusibility and penetrating power, and it is no doubt this portion combined with the fractions β and γ which gives the secretion its all-pervading property.

Lack of material has prevented the further study of the physiological properties of the secretion. I take the liberty, however, of presenting certain facts that I have gathered from various sources in regard to these properties, in the hope that they will be of general interest.

The substance is a powerful anæsthetic,‡ and has also been used as an antispasmodic. When inhaled without the admixture of a large amount of air the victim loses consciousness, the temperature falls, the pulse slackens, and, if the inhalation were prolonged, the results would doubtless prove fatal.

When recently ejected, the fumes from this liquid are overpoweringly pungent and extremely irritating * to the air passages, and are said to be capable of producing œdema of the glottis. It is also said to be an efficacious remedy in certain spasmodic affections of the air

* Liebig's *Annalen*, 239, 131.

† Victor Meyer u. Jacobsen, Lehrbuch der organ. Chemie, p. 225.

‡ Skunk Perfume as an Anæsthetic. *Virginia Med. Month.*, August, 1881 vol. viii, No. 5, pp. 359, 360.

passages. If any of this fluid finds its way into the eye it produces intense pain and sets up acute conjunctivitis, which commonly runs its course in a week or ten days. Moreover, we have reliable accounts of the entire loss of vision from this cause.

Swarts † complained while working with the secretion, especially when distilling with water vapour, of violent headaches and dysuria. His urine was voided with a burning sensation, and deposited a brown sediment which smelled like musk, as did also the urine. His perspiration for several days retained the odour of the secretion. Though I have worked with comparatively large quantities of the secretion and during a period of three months, I have failed to observe in myself the symptoms described by Swarts.

As far as the literature of this subject is known to me, the presence of the higher mercaptans in the animal organism has never before been demonstrated.

It is true that M. v. Nencki ‡ has found methyl mercaptan in the urine of those who had partaken freely of asparagus, and also among the gases given off during the putrefactive decomposition of the proteid; # and that methyl mercaptan has also been found in the urine as the product of a special bacterium,‖ and also among the gases of the large intestine; △ but all of these mercaptans, with the exception of the one arising from the use of asparagus, owe their origin to the putrefactive decomposition of proteids. The mercaptan in the skunk secretion, on the other hand, differs widely from the above, as far as its origin is concerned. It is without doubt a normal product of the cell activity of the gland.

The secretion of *M. mephitica* is not only remarkable for the quantity (about thirty per cent) of sulphur which it contains, and for this sulphur being in an unoxidized condition, but for the light it throws on the normal sulphur metabolism of the animal organism.

* Merriam, *op. cit.* † *Op. cit.*

‡ *Archiv f. exp. Pathol. u. Pharmakol.*, Bd. xxviii, pp. 206–209.

M. v. Nencki u. N. Sieber. *Monatsh. f. Chemie*, Bd. x, pp. 526–531.

‖ Karplus. Virchow's *Archiv*, Bd. cxxxi, pp. 210–222.

△ L. v. Nencki. *Sitzb. d. kais. Akad.* (Wien), Math.-Naturwiss. Classe, III. Abth., 98, pp. 437, 438.

Abel * has isolated from the dog's urine a substance (ethyl sulphide) closely related to those found in this secretion, and has expressed the belief, founded on certain experiments, that it is a normal product of cell activity and not a product of putrefactive decomposition in the intestine. My results supplement his and give another instance of a highly unoxidized sulphur compound which is formed in the breaking down of the proteid molecule, and which is protected in some way from oxidation, or if oxidized, is subsequently reduced.

It is useless to speculate on the immediate forerunners of this substance or substances with the little knowledge we at present possess. Further examination of this interesting secretion, and further knowledge of sulphur metabolism, are required before we can theorize with much profit. It would, however, be of general interest, and no doubt increase our knowledge in this direction, to examine the urine of the skunk and determine the condition of its sulphur contents, also to conduct a number of feeding experiments for the purpose of determining the changes in the sulphur contents of the secretion and of the urine.

I will say in closing that after completing my investigation of the secretion I intend to carry on experiments along the lines just referred to.

* *Zeitschr. f. physiol. Chem.*, Bd. xx, S. 253.

ON THE ACCELERATOR AND INHIBITORY NERVES TO THE CRAB'S HEART.

By F. S. CONANT
AND
H. L. CLARK.

PLATES XII AND XIII.

(*From the Physiological Laboratory of the Johns Hopkins University.*)

OBSERVATIONS on the nervous control of the heart in decapod crustaceans were published in 1880 by Plateau,* who worked upon *Macrura* and *Brachyura*, obtaining substantially the same results in both. His conclusions were that accelerator impulses reach the heart from the cerebral ganglion over the so-called "cardiac nerve," while inhibitory impulses have their centre in the ventral chain of ganglia and can not be aroused by stimulation of the cerebral ganglion. The course of the fibres from the central nervous system to the heart was not followed anatomically, but Plateau believed that the "cardiac nerve" arose from the cerebral ganglion and passed backward along the under side of the ophthalmic artery to the heart, while he supposed the inhibitory nerve to accompany the sternal artery into the pericardium. These observations and conclusions, so far as the *Brachyura* are concerned, have been directly contradicted by the recent work of Jolyet and Viallanes on the European crab, *Carcinus mœnas*.† They found no trace of the existence of the "cardiac nerve," and they located both inhibitory and accelerator centres in the anterior part of the thoracic ganglion. One of their most important results was the discovery on each lateral wall of the peri-

* F. Plateau. Recherches physiologiques sur le cœur des Crustacés décapodes, *Arch. de zoöl., vol. I.*

† F. Jolyet et H. Viallanes. Recherches physiologiques sur le système nerveux accélérateur et modérateur du cœur chez le crabe, *Ann. des sci. nat. zool.*, t. xiv, 1893.

cardium of a "ganglionic swelling" from which proceeded several nerves, evidently connected in some way with the action of the accelerator and inhibitory centres in the thoracic ganglion; but they did not trace out this supposed connection either anatomically or physiologically. As a supplement to their work, we undertook, at the suggestion of Professor Howell, a series of experiments on the American edible crab, *Callinectes hastatus*, which may be obtained in the markets of Baltimore throughout the greater part of the year. It may be said at once that so far as there are any disagreements between the work of Plateau and that of Jolyet and Viallanes, our observations have uniformly supported the latter. We have been unable to find any evidence of the existence of the so-called "cardiac nerve," and stimulation of the cerebral ganglion always gave reflex inhibition, which disappeared when the œsophageal commissures were cut. (Fig. 1, Plate XII.)

For a better understanding of the following experiments a brief description of the nervous system will first be given. As in all the *Brachyura*, the ventral ganglia in *Callinectes* are concentrated into the so-called thoracic ganglion, through the centre of which passes the sternal artery. The relation of the chief nerves to this ganglion will be readily seen in Fig. 7, Plate XIII. Attention is especially called to one pair (R. C.) arising on the dorsal surface just behind its anterior edge. These pass obliquely upward to the lining of the carapace and the roof of the gill chamber, and are known as the "recurrent cutaneous" nerves. Posterior to these there arise on each side three small nerves, which pass upward and backward to a plexus within the pericardium on the same side. This plexus corresponds to the "ganglionic swelling" and its nerves, figured by Jolyet and Viallanes, and will be spoken of in this paper as the pericardial plexus. Of these three nerves, the most anterior (Fig. 7, Plate XIII, *a*), leaving the ganglion with the recurrent cutaneous nerve, runs upward close beside it for a short distance, and then, turning abruptly, passes backward along the side of the body cavity to the pericardial plexus. The middle nerve (Fig. 7, Plate XIII, *b*) leaves the ganglion near the base of the nerve to the third maxilliped and, passing out

ward and upward, runs close beside the nerve just described, entering the plexus parallel to it. The third or posterior nerve (Fig. 7, Plate XIII, *c*) leaves the ganglion near the base of the first ambulatory nerve and, passing upward through a lobe of the liver, turns backward along the side of the body cavity and enters the plexus ventral and almost at right angles to the other two. The course and function of these three nerves were not worked out by Jolyet and Viallanes on *Carcinus*. While they figure all three entering the "ganglionic swelling," they make special mention only of the third, and of that they simply say that, passing into the substance of the liver, it loses itself in the direction of the thoracic ganglion.

In our work two methods of exposing the heart and nervous system were employed according to the experiment to be performed. Ordinarily the thoracic ganglion was laid bare by a large opening on the ventral side of the crab, as figured by Jolyet and Viallanes, while on the dorsal surface a portion of the carapace was removed just over the heart, the beat being thus recorded through the intact pericardium. The crab was then tied to a vertical frame so that the ventral surface was presented for stimulation, while the pericardium could be connected with the recording apparatus. This connection was made by a horizontal piece of pith to a delicate vertical tambour, which communicated by a rubber tube with a similar tambour placed horizontally. Upon the latter a pith upright transmitted the movements to a horizontal writing lever, which recorded upon a vertical drum.

In the second method, after exposing the nervous system as above, the heart was laid bare by cutting away everything on the ventral side down to the pericardium. The records were taken with the crab on its back by means of an upright resting on the heart and communicating directly with a horizontal writing lever.

It must not be supposed from the experiments given below that the response of the thoracic ganglion to electrical stimulation was always the same. Some crabs would give no acceleration until the inhibitory nerves were cut; others would give no inhibition so long as the accelerators were intact; with others, in poor condition, it was impossible to get either acceleration or inhibition satisfactorily.

Jolyet and Viallanes believed that the nerves *c* (Fig. 7, Plate XIII) carried both accelerator and inhibitory fibres, and they apparently supposed that the nerves *a* and *b* played no part in the nervous control of the heart. That such is not the case in *Callinectes* can be readily shown by cutting the nerves *c* at the plexus, after which acceleration and inhibition may still be obtained from the thoracic ganglion, while after cutting the nerves *a* inhibition can not be obtained from any point, even though the nerves *c* are intact. The following evidence is offered for the existence of separate inhibitory and accelerator nerves in *Callinectes*.

The Inhibitory Nerves.

Experiment I: showing the function of the nerves *a*.

Crab opened by the second method. Inhibition obtained by stimulation of the thoracic ganglion. The nerve *a* on each side cut inside the pericardium; no more inhibition from ganglion. The end of nerve *a* attached to plexus stimulated; prompt inhibition. (Fig. 2, Plate XII.)

Experiment II: showing physiologically that the nerves *a* enter the ganglion along with the recurrent cutaneous nerves.

Crab opened by the first method. All the nerves to the ganglion except the recurrent cutaneous cut, and inhibition still obtained from ganglion. Recurrent cutaneous nerves cut, and inhibition no longer obtained from ganglion, but produced at once on stimulation of the peripheral end of either recurrent cutaneous. (Fig. 3, Plate XII.)

Experiment III: showing that the nerves *a* are the only inhibitory nerves.

Crab opened by the first method. Inhibition obtained from ganglion. Recurrent cutaneous nerves cut. No further inhibition from ganglion, though all the other nerves were intact. Inhibition from peripheral end of recurrent cutaneous nerve, as in Experiment II.

Experiment IV: showing that the nerves *c*, which Jolyet and Viallanes believed to contain both the inhibitory and accelerator fibres, do not contain inhibitory fibres.

1. Crab opened by second method. Inhibition from ganglion. Nerves *c* cut inside the pericardium, and inhibition still obtained from ganglion. Nerves *a* cut inside the pericardium, and no further inhibition obtained except from stimulation of their peripheral ends.

2. (Converse of 1, on another crab.) Inhibition from ganglion. Both nerves *a* cut inside pericardium, and no further inhibition obtained except from their peripheral ends.

Cutting the inhibitory nerves (*a*) seems to have no effect on the heart beat except sometimes a very brief inhibition due to their stimulation; not the slightest evidence of their tonic activity was found.

THE ACCELERATOR NERVES.

Experiment V: showing the function of the nerves *c*.

Crab opened by the second method. Acceleration obtained from ganglion. Nerves *b* cut inside the pericardium; acceleration still obtained. Nerves *c* cut; no more acceleration from ganglion. Immediate acceleration from stimulation of the end of nerve *c* connected with the plexus. (Fig. 4, Plate XIII.)

Experiment VI: showing the function of nerves *b*.

Crab opened by the second method. Nerves *c* cut; acceleration still obtained from ganglion. Nerves *b* cut; no further acceleration from ganglion. Immediate acceleration from stimulation of the end of *b* connected with the plexus. (Fig. 5, Plate XIII.)

Experiment VII: showing that nerve *c* leaves the ganglion in the region of the first ambulatory nerve.

1. That *some* accelerator leaves at this place.

Crab opened by first method. All the nerves cut away from the ganglion except the first ambulatories; acceleration obtained from the ganglion. The first ambulatories cut; no more acceleration from ganglion.

2. That it is the nerve *c*.

Crab opened by second method. First ambulatory nerve on one side cut close to the ganglion and peripheral end stimulated; acceleration obtained. Nerve *c* on same side cut within the pericardium; no more acceleration obtained from peripheral end of first ambulatory nerve. Evidently nerve *c* was cut with the first ambulatory and remained attached to it.

Experiment VIII: showing that nerve *b* leaves the ganglion in the region of the third maxilliped nerve.

1. That *some* accelerator leaves at this place.

Crab opened by first method. First ambulatory nerves cut, the cut being made deeply to insure sectioning nerve *c*; acceleration still obtained from ganglion. Third maxilliped nerves cut deeply; no more acceleration from ganglion.

2. That it is the nerve *b*.

Crab opened by second method. First ambulatory nerves cut deeply; acceleration obtained from ganglion. Nerves *b* cut inside pericardium; no more acceleration from ganglion.

A very conclusive demonstration of the accelerator function of nerve *b*, as well as of the inhibitory function of nerve *a*, may be obtained by placing a pair of fine electrodes between the two nerves, and pressing upon first one and then the other. The marked acceleration given by *b* is followed immediately by inhibition on touching *a*, and *vice versa*.

That each of these three nerves to the heart leaves the ganglion as an independent nerve is shown by both anatomical and physio-

logical evidence. The accelerators (*b* and *c*) are much too delicate as they leave the ganglion to be seen in the living crab. In alcoholic material, however, they can be traced with the aid of a dissecting microscope and are found to preserve their independent course throughout. In the diagram (Fig. 7, Plate XIII) they are exaggerated in size, except at the plexus. Here they may be easily seen with the naked eye. The inhibitory nerves are larger, and by careful search can be seen in the living crab, running as separate fibres alongside the recurrent cutaneous nerves as they enter the ganglion. With the exercise of sufficient care the recurrent cutaneous, third maxilliped, and first ambulatory nerves can be cut without injuring the small nerves to the heart which accompany them. Ordinarily, however, cutting the first ambulatory nerves severs the one pair of accelerator nerves, and sometimes the other also, so that acceleration from the ganglion is no longer possible. In such cases stimulation of the peripheral end of the cut ambulatory gives acceleration usually (Fig. 6, Plate XIII), while stimulation of the process of the endoskeleton that lies just in front of the first ambulatory nerve on each side, about one centimetre from the ganglion, gives acceleration always. Both accelerator nerves pass over this process, and applying the electrodes to it evidently stimulates them.

As in the case of the inhibitory nerves, we found no evidence of tonic activity in either accelerator. In fact, the thoracic ganglion may be removed in part or in whole without apparently producing any effect on the rate of the heart beat. Evidently *Callinectes* differs from *Carcinus* in this respect, or else our material, not coming directly from the water, was less sensitive. It is worthy of remark that acceleration, as well as inhibition, is produced without any appreciable latent period, as examination of the figures will show.

Jolyet and Viallanes state that cutting the fibres of the plexus between the two branchio-cardiac orifices renders impossible all inhibition or acceleration, and they therefore conclude that it is the posterior branches that carry the inhibitory and accelerator fibres from the plexus to the heart. In *Callinectes* we have cut the plexus as far forward as the anterior orifice, and found no evidence of

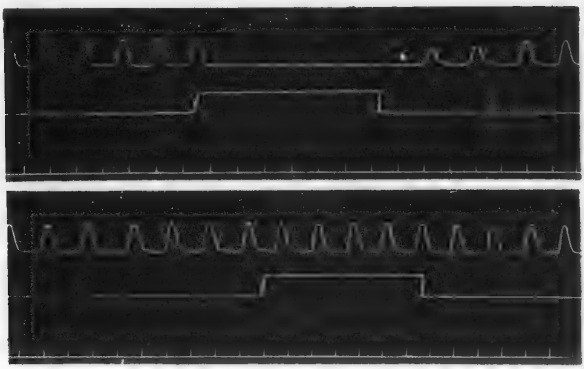

Fig. I.

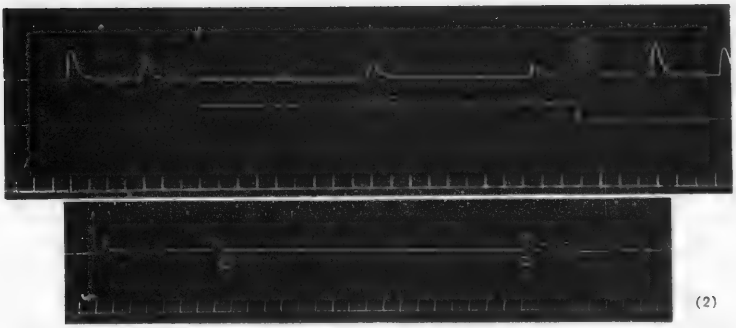

Fig. II.

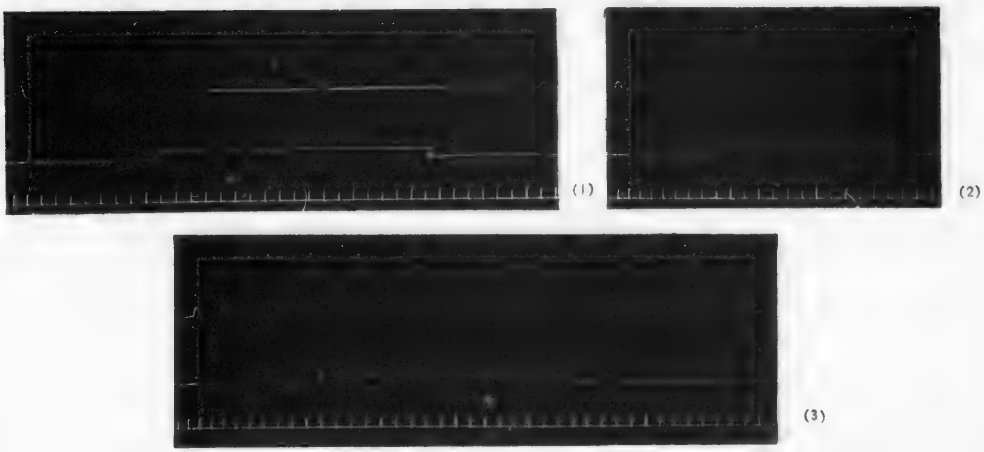

Fig. III

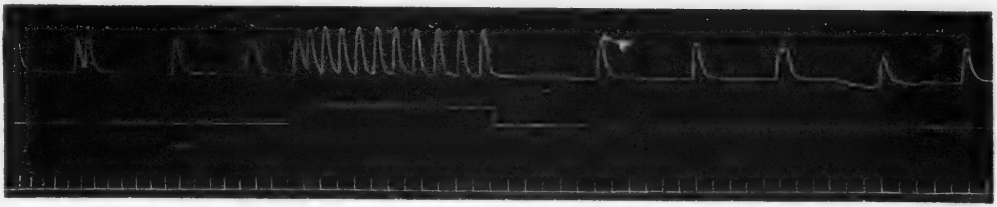

Fig. IV.

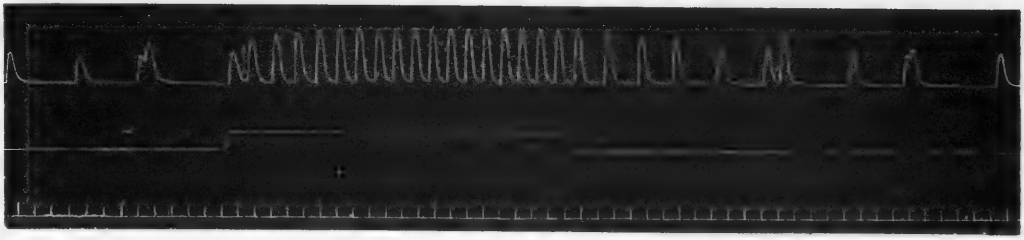

Fig. V.

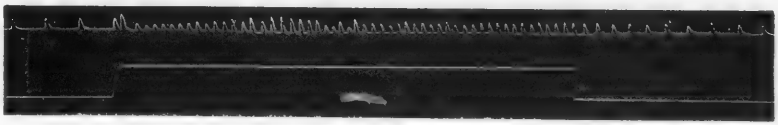

Fig. VI.

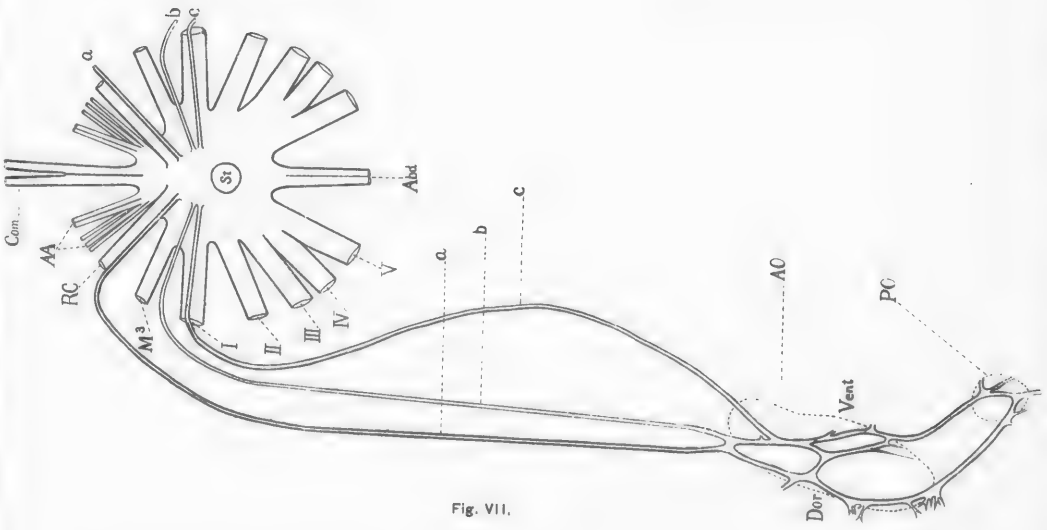

Fig. VII.

any effect on the action of the inhibitory or accelerator nerves, showing that at least the main course of the fibres from the plexus to the heart lies anteriorly.

Explanation of the Plates.

Plate XII.

Fig. 1.—(1) Reflex inhibition from stimulation of cerebral ganglion. (2) Cerebral ganglion stimulated after cutting the commissures to the thoracic ganglion. (The tracing to be read from left to right, as all the following. Time record in seconds.)

Fig. 2.—(1) Stimulation of nerve *a* near plexus. (2) The same from another crab, to show the complete inhibition and the improved beat following that were the usual results.

Fig. 3.—(1) Stimulation of thoracic ganglion. (2) The same after cutting the recurrent cutaneous nerves. (3) Stimulation of peripheral end of one of the recurrent cutaneous nerves.

Plate XIII.

Fig. 4.—Stimulation of nerve *c* near plexus.

Fig. 5.—Stimulation of nerve *b* near plexus.

Fig. 6.—Stimulation of peripheral end of one of the first ambulatory nerves, cut near the ganglion.

Fig. 7.—

Thoracic ganglion and left pericardial plexus of *Callinectes hastatus* (3 ×). The ganglion is drawn from the dorsal side. The plexus is laid over on its left side; in its natural position it lies in a plane at right angles to that of the ganglion. Com, œsophageal commissures, passing to cerebral ganglion; AA, nerves to the anterior appendages; RC, recurrent cutaneous nerves; M^3, to the third maxilliped; I, II, III, IV, and V, nerves to the legs; Abd, to the abdomen; St, sternal artery; *a*, inhibitory; *b* and *c*, accelerator nerves; AO, anterior branchio-cardiac orifice; PO, posterior branchio-cardiac orifice; Dor, dorsal; Vent, ventral regions of the plexus.

A CASE OF CIRCUMSCRIBED UNILATERAL, AND ELECTIVE SENSORY PARALYSIS.

By LEWELLYS F. BARKER,

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(*From the Physiological Laboratory of the University of Leipsic.*)

By elective sensory paralysis is to be understood one in which certain only of the functions of the sensory nerves are abolished. The observations which are to be briefly reported here have been made upon my own left arm, and in large part by myself. The anamnesis is as follows: I am at present twenty-eight years old, and, as far as I remember, aside from the ordinary diseases from which children suffer, I have always been tolerably healthy. For the past nine or ten years I have noticed at times along the inner (medial) side of the left arm radiating pains, which are prone to appear on standing for a long time or after a long walk, especially when I am overtired or a little run down in health. For about the same length of time, sensations of numbness and formication have made themselves noticeable. These have been referred to the same area, and have affected also, in a slight degree, the little finger and the ulnar side of the ring finger of the left hand. The pains, which, as a rule, are not very severe, can be quickly stopped, or essentially relieved, by lying down or by elevating the left shoulder. Beyond these symptoms I have nothing to complain of, and during the past two or three years unpleasant sensations of any kind have rarely existed. The motility of the left arm is in no way diminished or interfered with. This arm is slightly weaker than the right, which is easily to be explained by the fact that it has been used less. Alterations in the muscles or the skin have never been perceptible. The secretion of sweat is normal, and goose

skin occurs on the left as often and to the same extent as on the right side.

About a year ago the arm was examined by my friend Dr. H. M. Thomas, neurologist at the Johns Hopkins Hospital, and the presence of a sharply circumscribed zone of anæsthesia for temperature and pressure was demonstrated in the area mentioned.

The last cervical vertebra shows on the left side a rigid, bony process apparently firmly attached to it. This process projects above the clavicle in front where it can be easily seen and felt. It is extremely little if at all movable, and it accompanies the first rib for a short distance, lying directly above it. This process is probably to be looked upon as a cervical rib.

Being in Leipsic during the spring and summer of 1895, at the suggestion of Prof. v. Frey, in the Physiological Laboratory there, I undertook an exact investigation of the conditions in the arm, in order to determine the extent and the kind of disturbance of sensibility qualitatively, and, as far as possible, also quantitatively. Unfortunately, my time did not permit me to make the study complete for all parts of the arm, and the investigations were therefore limited to the changes in the forearm, an attempt being made to establish with extreme care the character of the anæsthesia in this region. Although the working out of the whole zone must be deferred until later, I am already in a position to state that the sensory changes in the anæsthetic areas of the upper arm and of the hand (the deviations from the normal in the latter are very slight) resemble exactly in all essential points those in the forearm.

Methods.

The sensation for cold, warmth, pressure, and pain was examined, and for these four sense-qualities the limits of the anæsthetic areas were at first tolerably accurately established by quick methods of examination, and later the distribution of the different sense-points and their threshold-stimuli (*Reizschwellen*) were carefully determined, with exact methods of localization and measurement.

I. *Cold-sensation.*—For quick limitation, metal rods with an end surface of one square centimetre or a camel's-hair brush moistened with ether were used. To localize the individual cold-points there were employed metal rods with conical ends, as in Goldscheider's thermæsthesiometer,* and fine steel pens moistened in water at the room temperature; finally, for extremely exact localization, pieces of copper wire of 0·2 millimetre diameter were used. I prepared a large number of such bits of copper wire, varying in length from a half to six millimetres, mounted on little sticks of wood surrounded, except at one free end, by sealing-wax. By means of these the investigator is in a position to make quantitative examinations by which the more or less accurate estimation of threshold-values in "heat-quantities" is rendered possible. If the temperature of the skin is known, through the application of water which is felt neither as warm nor as cold, and also the temperature of the room in which the wires are used, one can obtain the "heat-quantity" which a given bit of wire can yield to the skin or take from it by multiplying together (1) the difference between the above temperatures; (2) the "heat-capacity" of copper; and (3) the "mass" of the piece of wire.

II. *Warmth-sensation.*—The hot tube of Blix,† provided with a thermometer, was used almost exclusively, since either the broad surface or the fine point, as desired, can be applied to the skin. An attempt was also made to stimulate the warm points with known "heat-quantities," the pieces of copper wire previously described being heated to 40° C. in an air-bath.

III. *Pressure-sensation* (*Druckempfindung*).—For the first examination a soft pencil, or, still better, a little wad of absorbent cotton, was drawn over the skin, especially over the hairs. For finding the pressure-points, and particularly for determining their threshold-values, we used punctiform faradic stimulation as well as the test-hairs (*Reizhaare*), finely graded, according to the pressure they

* Du Bois' *Archiv., Suppl.*, 1885, p. 2.
† *Zeitschr. f. Biologie*, Bd. xx, 1884, p. 153.

exerted. These test-hairs were first devised and used by Prof. v. Frey.*

IV. *Pain-sensation.*—For this purpose also test-hairs similar to those used in examining the pressure-sense, only of higher pressure-values, were employed, but an extensive use was also made of other mechanical as well as of electrical and thermic stimuli.

Since an alteration of the sensory conditions during the course of the investigation was not to be expected, and, as a matter of fact, could not be demonstrated, the test was carried through in a systematic way for each single one of the sense-qualities under examination, after which the next kind of sensation was taken up for study. The examination of each kind of sensation was divided into three stages. At first the limits of the anæsthetic area for the sense-quality concerned were broadly determined by one of the investigators named below and marked by coloured lines. After this, in numerous sittings, the sense-points were carefully sought out one by one and marked by myself. Finally, the results were confirmed, for the thermic sensations by Dr. F. Kiesow, for pressure- and pain-sensation by Prof. v. Frey. It is a great pleasure to me to heartily thank once more both these gentlemen for the repeated and most painstaking confirmatory tests which they so cheerfully made.

Every one who has had any experience in seeking out sense-points knows that the examination even of small areas of the skin, if it pretend to completeness, can not be terminated in one sitting. Estimations of the number and position of sense-points are also the more reliable the less the stimuli exceed the threshold-value; if one acts accordingly, the testing makes such claims on the attention that the examiner is incapable of making any one sitting very prolonged. Inasmuch as the examination in this case had to include tolerably large areas of the skin, it became necessary to establish the position of the sense-points which had been sought out and confirmed in some more permanent way than could be arrived at by any kind of colour-

‡ Beiträge zur Sinnesphysiologie der Haut, i, ii, iii, Leipzig, 1894–'95. Aus den *Berichten der math.-phys. Classe der K. Sächs. Gesellsch. der Wiss.*, July 2 and December 3, 1894, and March 4, 1895.

ing of the point upon the skin. This problem was solved in the following manner: The skin was divided into a number of fields according to the course of the visible cutaneous veins and also with regard to certain irregularities, which have yet to be mentioned, in the lines limiting the anæsthetic zone. As soon as the determination of the sense-points of one quality for such a cutaneous field had been completed, a piece of gelatin-paper was laid upon the skin and the sense-points and the limits of the area were accurately copied and transferred to a plaster-of-Paris cast of the whole arm. For each quality of sensation examined a special plaster cast was made. This method of keeping track of the topographical relations of the investigation proved to be particularly serviceable. With the help of these casts it has been possible to find again very quickly any desired single sense-point.

General Results.

It appears to me desirable to state first generally the main results of the investigation, and later to refer more in detail to the experiments which bear on the single sense-qualities.

1. The disturbance is limited to the domain of the nervi cutanei brachii et antibrachii mediales of the left arm.

2. In this area the sensation for warmth, cold, pressure, and touch (*Druck und Berührung*) are absent. In addition, no tickling sensation can be called forth in the cutaneous region concerned. The sensation for pain is apparently intact on superficial examination, but with accurate testing a slight diminution in the number of pain-points can be demonstrated.

3. The limit of the area of sensory disturbance on the flexor surface of the forearm is sharply and regularly defined, and is situated almost exactly in the middle between the radial and ulnar side. On the extensor side the limit is not sharply defined, and indeed is very irregular—i. e., the anæsthetic zone invades by deep indentations the normal area, and the transition from regions where the three sense-qualities are entirely absent to areas where sense-capacity is complete is very gradual. These findings can also be described in the follow-

ing way: On the *flexor surface*, passing from the radial to the ulnar half, the sense-points for warmth, cold, and pressure, which on the radial side are present in normal numbers, suddenly cease when we advance beyond the above-mentioned line.* In passing over the ulnar margin of the forearm *to the extensor surface*, single sense-points here and there again appear, increasing gradually in numbers toward the radial margin, but not being present in normal numbers until this margin is nearly reached.

4. The limits described, although they hold in general for each of the sense-qualities which are disturbed, show in detail many variations. These are in part to be accounted for by the fact that the distribution of the sensory points within the partially anæsthetic area follow the same rule as on the normal skin, namely, that of the three kinds of sense-points—pressure-points, cold-points, and warm-points—the pressure-points occur in greatest and the warm-points in smallest numbers. In addition to this, single sense-points or groups of sense-points of one kind are to be found located in places where none of the other sense-qualities is represented.

5. The sense-points of the partially anæsthetic area, whether they are isolated or mixed with others, as well as the points on the border of the normal and anæsthetic skin, react to normal threshold-stimuli. I can assert this absolutely for the pressure- and pain-points from numerous determinations of threshold-stimuli. The individual cold-points and warm-points show considerable differences in excitability, but these differences do not appear to be more marked than in normal areas.

Some Special Observations.

A. *Regarding Temperature-sensations.*—The excitability of the cold- and warm-points is, when fatigue is completely excluded, distinctly dependent upon the absolute temperature of the skin; with higher outside temperatures, after bodily exercise, and after meals, the excitability on previously clothed portions of the skin is greater, and the localization of the sense-points is made easier. Strong tem-

* Compare below the results regarding the pressure-points.

perature stimuli, even when they are of small area—the so-called punctiform stimuli—spread out to a considerable distance beyond the direct spot of skin to which they are applied. In the anæsthetic zone single demonstrable sense-points can be excited from a distance, although not with equal ease from all directions. One can probably accept the view that the direction of the blood stream and the course of the blood vessels in the skin are of essential significance as regards this point. The response to the stimulus in such cases occurs relatively late. The smallest amount of heat, the subtraction of which I have found could lead to the reaction of a cold-point, was 0·0024 calorie, or the same amount of heat by means of which the temperature of 2·4 cubic millimetres of water can be raised through 1° C. In this test direct mechanical excitation of the cold-points, which had previously been most exactly located, was excluded. Of twenty cold-points examined, there were eighteen threshold-values which lay between the amount of heat just mentioned and an amount twelve times as great. I have made determinations of the threshold-stimuli for the warm-points only in single instances. These in relation to those of the cold-points are relatively high—a fact which may stand in some relation to the long latent period which is, as is well known, characteristic of the warm-points. The question needs and deserves a much more thorough study than I could devote to it in view of the work which had to be done in connection with the main portion of this investigation.

The paradoxical cold sensation (v. Frey *) was examined on some fifty cold-points by Dr. Kiesow; the majority of these yielded, on the application of temperatures of from 40° to 50° C., exclusively the sensation of cold. In some few, cold and warmth were felt at the same time; in the case of some others, cold was felt at first, and after some time the sensation of warmth appeared. In the latter instances warm-points were always demonstrable in the immediate neighbourhood of the cold-points. The paradoxical excitation appeared even at temperatures which were absolutely unassociated

* Cf. v. Frey, *op. cit.*, iii, p. 172.

with any painful quality, a point upon which especial emphasis must be laid.

The reverse experiment—the stimulation, by the application of bits of ice, of isolated warm-points lying in the anæsthetic zone—never succeeded. If one allowed, however, a drop of ether to evaporate over such a warm-point, after some time a slight feeling of warmth resulted. Here we had to deal probably with a reaction on the part of the blood vessels. I have convinced myself many times of the electrical and mechanical excitability of the temperature-points, although these modes of excitation were not made the subject of an especial study. I have, however, confirmed the results of Blix and Goldscheider in this connection.

B. *Pressure-sensation.*—The area of complete anæsthesia to pressure is smaller than that to thermic stimuli; it never reaches beyond the ulnar margin of the forearm on the flexor surface. As Prof. v. Frey * has found, the spots where the pressure-threshold is lowest (*Orte niedrigster Druckschwelle*) exhibit a very definite relation to the hair follicles. There is no perceptible difference in the number or in the development of the hairs on the anæsthetic and the normal skin, although in the former almost all the hair follicles are non-responsive to stimuli of pressure or touch. Between the completely anæsthetic and the normal area lies a transitional zone in which some of the hair follicles are still sensitive. The transitional zone is, on the flexor side, scarcely one centimetre in width, whereas on the ulnar side it includes the ulnar margin and the greater part of the extensor surface. That these more or less isolated pressure-points possess normal threshold-values has already been mentioned. The hair follicles which do not react to pressure fail to yield the characteristic oscillatory or buzzing sensation on faradic stimulation. (Cf. v. Frey, ii, p. 291.) As a special peculiarity of the isolated pressure-points I might state that with increase of the mechanical stimulus the sensation gains only a little in intensity. This would appear to show that the deformation of the skin increasing with the stimulus is recognised more by the number of the

* Cf. v. Frey, *op cit.*, i, p. 190; ii, p. 287.

sensory points affected than by the intensity of the excitation of the single-points.

While stroking the hairs of an uninjured spot of skin calls forth intense sensations of touch or even of tickling, within the anæsthetic area such a stimulus is not perceived at all. Mechanical influences which affect the skin itself become recognised as soon as the deformation, displacement, or pulling of the skin extends beyond the limiting lines into the area in which the pressure-points are sensitive.

C. *Pain-sensation.*—Pain-sensation can be called forth within the anæsthetic zone on very many spots. However, exact investigation shows that the points especially set apart for this sensation, the pain-points, do not everywhere lie so closely together as in normal areas. One can find on stimulation with the very finest needles or by punctiform faradization painless spots of the extent of more than several square millimetres; areas of this size not sensitive to pain do not occur normally in the region under examination. Nevertheless, the disturbance of the pain-sense is everywhere but little marked, as compared with that of the other sense-qualities, so that on superficial testing one might arrive at the conclusion that he had to deal with an anæsthesia of all the qualities of sensation with the exception of pain.

A series of influences which in normal regions at first evoke other sense-qualities and only subsequently give rise to pain-excitation, here are felt exclusively as painful. Pricks with a fine needle excite only a feeling of pain without calling forth previous touch- or pressure-sensations, and are answered by lively reflexes. A small piece of ice laid on the anæsthetic zone is at first not felt at all, and only after the lapse of a certain time excites a sensation of pain. Metal at the temperature of 47° C. and upward very quickly causes pain, though there is absolutely no accompanying sensation of warmth. Here the opportunity is offered of observing the so-called "temperature-pain" wholly independent of the temperature-sensations which on the normal skin accompany it or precede it.

As to the "heat-pain," it was shown that the threshold-stimulus

for it lies at a different level in different places. On some points a painful response was obtained to a temperature of 46° C.; this, however, was rare. For the most part, pain was felt first at higher temperatures, and hardly ever under one of 50° C. These experiments are all in favour of the view that the painful sensations which under similar conditions are felt on normal portions of skin have their origin in the stimulation of the organs directly concerned in pain-sensation. Under ordinary conditions the sensations of pressure, heat and cold, which are normally always previously evoked, act to a certain degree as protective mechanisms which very often suffice to enable one to avoid the pain-sensation. Within my anæsthetic zone these introductory sensations are absent, and I became, so to speak, surprised by the unannounced appearance of pain when efficient stimuli, though not strong enough nor so widespread as to involve the area which possesses normal sensation, were applied. This may perhaps afford an explanation of the fact that I have throughout life preferably used the right arm relatively more than most right-handed men, since there has been a partly conscious and partly instinctive attempt to protect the left arm.

The threshold-stimulus for mechanical excitation of pain I have found, by testing with Prof. v. Frey's test-hairs in the anæsthetic area, to vary within the same limits as in normal areas. For the "heat-pain" I found the region of the wrist and the ulnar border of the forearm more sensitive than those areas which lie toward the fossa cubiti. In addition, it must be remarked that exactly in the last-mentioned region the diminution of the pain-points is most distinctly to be made out.

The capacity to localize is very incomplete within the area where pain exclusively is felt. The exact situation of compass points is appreciated only very inaccurately. In applying the compass test of Weber the neighbourhood of the normal domain must be avoided, and in addition one must have made sure that the spots of skin struck by the two points actually possess pain-sensation. One finds then that pain-points which are separated from one another by as much as 10 centimetres can often not be distinguished.

D. *Motility.*—In conclusion I desire also to mention the results of an examination of the motility of both my arms which Privatdocent Dr. Windscheid was good enough to make for me.

Circumference in Centimetres.—The middle of the upper arm, R. 25; L. 24. The middle of the forearm, R. 21; L. 20.

Crude strength of left biceps and muscles of forearm weaker than right. In the faradic and galvanic excitability of the biceps as well as in conduction-resistance no distinct difference can be made out between the right and the left side. The contractions are never sluggish. Atrophy of single muscles is nowhere demonstrable.

Epicrisis.

In reviewing the disturbances described, they are seen to be essentially "phenomena of absence," a number of nervous functions which are peculiar to the normal skin not being present. Irritation-phenomena, as far as they can be observed, are by no means prominent. With this conforms the observation that an alteration of the threshold-values of the retained functions in the partially anæsthetic area could nowhere be distinctly demonstrated.

One will find, in considering the etiology, in the purely unilateral seat of the disturbance and in the definite limitation to two cutaneous nerves, strong ground in support of the view that the cause of the disturbance is peripheral, and it is probably to be sought in the cervical rib, which is almost certainly present. Quite recently attention has been several times drawn to the fact that the presence of a cervical rib through pressure on the brachial plexus can give rise to disturbances of sensibility and motility. The cases in which the symptoms have disappeared after resection of the rib are most convincing in this connection.* Since in the case just described a loss in motor capacity is not demonstrable, the disturbance appearing to be much more of a purely sensory nature, one might be inclined to look for the seat of the lesion in the nerve roots or in the spinal cord. In opposition to this, however, it may be pointed out that at the site of

* The literature will be found appended to the article by Tilmann in the *Deutsche Zeitschrift für Chirurgie*, Bd. xli, S. 330.

the disturbance the sensory paths must already be arranged with regard to the peripheral distribution, if one is to explain the fact that the two above-mentioned cutaneous nerves alone have been injured. Now, according to Henle, the nervi cutanei brachii et antibrachii mediales arise from the lower cord of the plexus brachialis which represents a continuation of the eighth cervical and first thoracic nerves, and thus it is at any rate possible that the nerves mentioned could be affected by a cervical rib.

Most interesting in the case before us is the slight amount of disturbance of pain-conduction as compared with that of the other sensory functions. However, even this finding represents only one of the numerous variations which are possible in elective injuries of peripheral nerves. As regards this point, numerous experiences could be referred to, more particularly the newer observations of Cavazzani * and the younger Charcot.† In Charcot's case, which is antithetic to mine, the individual, as the result of a scar in the cubital region, suffered from a paralysis of temperature- and pain-sensations in a large part of the hand, while the sensations of pressure and touch were retained. The loss of temperature- and pressure-sensations was unequal in the case observed by Cavazzani. These two cases, to which others might be added, are the more convincing in that after excision of the scar in the one instance and the healing of the wound in the other the sensory disturbances disappeared. As physiological experiments have shown that the nerve fibres of one portion of a limb—for example, those for the flexors and extensors—are not evenly distributed within the nerve trunk leading to it, but are already arranged according to their functions, so it would appear that a similar arrangement may hold also for the sensory nerves with regard to the different paths which they are to follow within the spinal cord. Thus, whereas in the motor nerves the fibres appear to be arranged according to the muscles to which they are to be distributed, for the sensory fibres we may have to consider rather

* *Archives ital. de Biologie*, t. 17, p. 413.

† *Comptes rendus de la Soc. de Biologie*, December 10, 1892.

the different paths which they have to follow on entrance into the spinal cord.

In accordance with these views the case just described would have to be looked upon as one in which the majority of the pain-conducting fibres have been retained, while the others in great part have disappeared, for some areas of skin even completely. In any case it appears to me that this view agrees better with the facts than the other that the nerves are retained, but that their excitation is no longer felt except in giving rise to pain. When one sees that in the periphery certain only of the sense-points have disappeared—for example, the pressure-points arranged in connection with the hair follicles, while the far more numerous pain-points are present in normal or in only slightly diminished numbers—one can scarcely avoid the conclusion that there are specific nerve-endings and nerve-paths involved, which have actually disappeared.

Further, the irregular limitation of the anæsthetic area and the occurrence of sense-points singly or in groups on the extensor surface of the forearm, seems to be worthy of mention. From this alone one might almost conjecture that, even normally, the endings of different sensory nerves are associated with one another in the skin in the most complicated manner. It is true, however, that one might think also of regenerations, and more especially since such have recently been observed by Fedor Krause * in the distribution of the trigeminus. But the adoption for the present case of either of these views would be associated with difficulties, considering the relatively very sharp limitation of the anæsthetic area on the flexor side.

In conclusion, I beg to tender my most sincere thanks to Prof. v. Frey, at whose suggestion and under whose supervision this research was carried out. Without his assistance I could hardly have appreciated fully the physiological bearings of the results obtained.

* *Münch. med. Woch.*, 1895, Nos. 25–27.